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野菜用アマランスにおける遺伝的多様性、 葉の色素沈着および環境ストレス耐性に関する研究

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Genetic Diversity of Vegetable amaranth

1.1.1 Genetic variation and diversity for morphological and nutritional traits

Vegetable amaranth serves as an alternative source of nutrition for vegetarian people in developing countries where the bulk of the population has little access to protein rich food. It contains high amount of protein with nutritionally critical amino acids, lysine and methionine, dietary fiber, dietary minerals and antioxidant compounds like ascorbic acid and beta-carotene [6, 7]. Recently, the genus has been reported to have medicinal value including anticancer properties [8]. It has been rated equal or superior in taste to spinach and is considerably higher in protein (14 - 30% on dry weight basis), minerals (Fe, Mn and Zn) and antioxidants like beta-carotene (90 - 200 mg/kg) and ascorbic acid (about 28 mg/100 g) compared to any other leafy vegetables [3, 6, 9-10]. Antioxidants like carotenoids, ascorbic acid, Fe, Mn, and Zn contents in vegetable amaranth are considerably higher than in many leafy vegetables [11-14]. Some metalloenzymes like catalase (Fe) and superoxide dismutase (Mn and Zn) required Fe, Mn and Zn minerals for their antioxidant activity [16].

The main vegetable type of amaranth, *Amaranthus tricolor* L., seems to have originated in South or Southeast Asia [1] and then spread through the tropics and the temperate zone [2]. Leafy vegetables are a valuable part of the diet owing to their nutritive values which plays an important role in the human diet [3, 4]. Among 60 species, vegetable amaranth (*Amaranthus tricolor*) is now very popular as vegetable in many Asian and African countries. In Bangladesh *Amaranthus tricolor* is grown year-round and it is the only crop available in the hot summer months when no other foliage crop grown in the field [5].

Generation of oxygen radicals, such as superoxide radical ($O_2^{\bullet-}$), hydroxyl radical (OH•), and non-free radical species such as H_2O_2 and singlet oxygen ($^1O_2$), is associated with cellular and metabolic injury, accelerated aging, cancer, cardiovascular diseases, neurodegenerative diseases, and inflammation [16]. Antioxidant neutralizes or removes free oxygen radicals in the body and helps to protect many diseases including cancer, cardiovascular diseases, neurodegenerative diseases and inflammation and prevent aging [8]. It has high adaptability under varied soil and agro-climatic conditions and great amount of genetic variability and phenotypic plasticity [17, 18]. It is also extremely adaptable to harsh environmental conditions, including high temperature and drought, and resistant against major

diseases [19]. Although vegetable amaranth is used as a cheap source of a variety of antioxidants, nutrient, little efforts have been made for its genetic improvement of this underutilized crop plant [4,19]. A large number of studies are available on genetic variability and interrelationships among various traits such as growth, nutrient contents, and antioxidants in many other crops [20-22]. However, reports on vegetable amaranth are rare [23].

A plant breeding program can be divided into three stages, *viz.* building up a gene pool of variable germplasm, selection of individuals from the gene pool and utilization of selected individuals to evolve a superior variety [24]. The available variability in a population can be partitioned into heritable and non-heritable parts with the aid of genetic parameters such as genetic coefficient of variation, heritability and genetic advance [25]. Correlation coefficient helps to identify the relative contribution of component characters towards yield [26]. The correlation between yield and a component character may sometimes be misleading. Thus, splitting of total correlation into direct and indirect effects would provide a more meaningful interpretation of such association. Path coefficient, which is a standard partial regression coefficient, specifies the cause and effect relationship and measures the relative importance of each variable [27]. Therefore, correlation in combination with path coefficient analysis will be an important tool to find out the association and quantify the direct and indirect influence of one character upon another [28]. Genetic diversity assessment is very useful tools that help a breeder to identify diverse parental combinations for creation of segregating progenies with genetic variability. It also facilitates introgression of desirable genes from a diverse germplasm into the existing genetic base population [29].

1.1.2 Variability in antioxidant leaf pigmentation and total antioxidant capacity

Vegetable amaranth serves as an alternative source of nutrition for people in developing countries since it is a rich and inexpensive source of mineral, vitamins, protein, dietary fiber, flavonoids, polyphenols, antioxidant leaf pigments like betalain, carotene, and chlorophyll [6, 12].

Coloring food products have been put forward in recent years as they considerably affect the acceptability of foods and are fundamentally linked to multisensory interactions including perception of flavor and significant enjoyment of food. The growing interest of consumers in the aesthetic, nutritional and safety aspects of food has increased the demand for natural pigments such as chlorophyll, betalain and carotene. Betalain are water-soluble compounds found in a limited number of families of the plant order Caryophyllales like *Amaranthus* have a unique source and important free radical-scavenging activity [30, 31].

Betacyanin are red to purple colored betalain (absorbance ranging from 530 to 545 nm and condensation of betalamic acid and cyclo-Dopa, considering hydroxycinnamic acid derivatives or sugars as residue) and yellow colored betalain known as betaxanthin (absorbance ranging from 475 to 485 nm and imine condensation products between betalamic acid and amines or amino acid residues) [32-37]. Similarly, carotene grouped into alpha-carotene, beta-carotene and xanthophyll. They are hydrophilic nitrogenous secondary metabolites which replace anthocyanins in the flowers and fruits of most plants in families of Caryophyllales. Betacyanin, betaxanthin and carotene are also free radical scavengers (antioxidants) [35, 38], which play an important role in human health. Their pharmacological activities include anticancer, [39-40] antilipidemic [41] and antimicrobial [42] activities, indicating that betalain and carotene may be a potential source for the production of functional foods. Presently, the only commercial source of betalain and carotene is the red beet root. The colorant preparations from red beet root labelled as E-162 are exempted from batch certification. E-162 is used in processed foods such as dairy products and frozen desserts [34].

Among the naturally occurring vegetable pigments, betalain are rare and limited to a few edible vegetables such as red beet and amaranth, while chlorophylls are widely distributed in plant species [43]. The active ingredients of betalain and carotene provide anti-inflammatory property to our food and act as potential antioxidants and reduce the risk of cardiovascular disease and lung and skin cancers and is widely used as additive for food, drugs, and cosmetic products because of natural properties and absence of toxicity [12, 44-46]. In Asia, and Africa vegetable amaranth is intake by boiling, making curries while in Americas, Japan, few Asian and European countries it is freshly intake by making salad or juice. Recently, we extracted red color juice for natural drinks containing leaf color pigments chlorophyll, betalain, and carotene from *Amaranthus*. It demands more genotypes enriched with leaf pigments.

1.1.3 Phenotypic divergence for antioxidant profile, nutritional and agronomic traits

Antioxidant vitamins, minerals and leaf pigments, phenolic compounds and flavonoids protect the body from harmful free radicals such as superoxide, hydroxyl, hypochlorite, hydrogen peroxide, lipid peroxides and nitric oxide. Free radicals can cause damage to cells and impair the immune system and lead to infections and various degenerative diseases like cancer, cardiovascular diseases, atherosclerosis, arthritis, cataracts, emphysema, retinopathy, neuro degenerative diseases and inflammation and prevent aging [8, 35, 38, 47-51]. Antioxidant vitamins and minerals include vitamins A, C, and E; beta-carotene; and the minerals selenium, zinc, manganese, copper, and iron [52, 53]. Antioxidant leaf pigments includes, betacyanin,

betaxanthin, chlorophyll, carotenoids [38]. Sufficient delivery of the first line defense antioxidants (Cu, Zn, Fe and Mn) from diet is required in order for the body to synthesize antioxidant metalloenzymes such as catalase (Fe) and superoxide dismutase (Cu, Zn, and Mn) [52]. Free radical scavengers include vitamin C, beta-carotene, and flavonoids and are considered to be second-line defense antioxidants [52]. Some metalloenzymes such as catalase and super oxide dismutase required Fe, Mn, Cu and Zn for their antioxidant activity [15, 52, 54].

Amaranths are C_4, dicotyledonous herbaceous plants that include approximately 70 species, of which 17 species produce edible leaves and three produce food grains [55]. The edible amaranth is a popular leafy vegetable in the South East Asia and is becoming increasingly popular in the rest of the continent and elsewhere due to its attractive leaf color, taste and nutritional value. *Amaranthus tricolor* leaves are a rich and inexpensive source of dietary fiber, proteins, vitamins and a wide range of minerals, leaf pigments, phenolic compounds and flavonoids [3, 4, 6, 16].

Genetic diversity assessment is a useful tool to help breeders for identifying appropriate parental combinations for the creation of suitable segregating progenies with excellent genetic variability that also facilitates integration of desirable genes from a diverse germplasm into the existing genetic base population [29]. Multivariate statistical methods have been successfully used to classify both quantitative and qualitative variation in many crop species, including mustard, [56] Russian wild rye [57], *Arachis* [58] and Ethiopian mustard [59]. There are few reports on genetic diversity in grain amaranth [60-62] performed a diversity analysis on *Amaranthus tricolor* for nutrient content and agronomic traits.

1.2 Abiotic Stress Response of Vegetable Amaranth

1.2.1 Response of nutrients, antioxidant phytochemicals, phenolic acid, flavonoid and antioxidant activity to drought stress

Natural antioxidants, in vegetables, have gained the attention of both researchers and consumers. Vegetable amaranth (*Amaranthus tricolor*) is a good source of minerals, vitamins, phenolics, and carotenoids; it also contains betalain, a nitrogen containing group of natural pigments, as well as proteins and fibers [6, 35]. Those secondary metabolites or natural antioxidants are involved in defenses against several diseases like cancer, atherosclerosis, arthritis, cataracts, emphysema, and retinopathy, neuro-degenerative and cardiovascular diseases [6, 35, 50].

The degree of damage by reactive oxygen species (ROS) is highly related to the balance between ROS production and its removal by the antioxidant scavenging system [64]. On the other hand, it has been reported that the plant cell membrane was more sensitive to rapid damage and leakage under water stress [64]. Plants can synthesize some secondary metabolites i. e., α-tocopherol (vitamin E), and polyphenol to protect them against oxidative damage caused by environmental stresses [65, 66]. These compounds evolve to detoxify reactive oxygen species in plants, but they also show beneficial activity against some human diseases related to oxidative damage and aging [67].

Amaranths are often described as drought tolerant plants [68, 69]. *Amaranthus tricolor* is a versatile food crop exhibiting high adaptability to new environments, even in the presence of different biotic and abiotic stresses [70]. The amount of metabolites in plants might be affected by different factors such as biological, environmental, biochemical, physiological, ecological, and evolutionary processes [71]. Among these factors, drought stress can highly enhance the concentration of secondary metabolites [72].

There are few reports related to the effect of water stress on secondary metabolites of different crops including leafy vegetables. To date, scarce information is available for betalainic food crops under water stress, although betaxanthin and betacyanin have recently attracted attention for their antioxidant activities [73]. Water stress elevated secondary metabolites such as beta-carotene content in Choysum in dry season trial [74], in perennial herbaceous [75], ascorbic acid in tomato [73], TPC, TFC in buckwheat [76], TPC, TFC and antioxidant activity in *Achillea* species [77]. In contrast, water stress reduced the protein content in buckwheat [79], beta-carotene content in Kailaan in dry season trial [74], ascorbic acid, Ca, Fe and Zn content [74].

1.2.2 Drought stress effects on growth, ROS markers, compatible solutes, non-enzymatic antioxidants

Amaranthus tricolor L. is one of the most important and popular leafy vegetables in Bangladesh including Southeast Asia, Africa and South America often cultivated in arid and semiarid regions with drought stress. Vegetable amaranth is the inexpensive sources of natural antioxidants like, vitamins, phenolics, flavonoids and a unique source of betalain (betacyanin and betaxanthin). These secondary metabolites or natural antioxidants are involved in defense against several diseases like cancer, atherosclerosis, arthritis, cataracts, emphysema, and retinopathy, neuro-degenerative and cardiovascular diseases [35, 48]. *Amaranthus tricolor* is often described as drought tolerant plants [68].

Drought stress leads to the accumulation of reactive oxygen species (ROS), which might initiate destructive oxidative processes such as lipid peroxidation, chlorophyll and betalain bleaching and protein oxidation. Plants have evolved both enzymatic and non-enzymatic defense systems for scavenging and detoxifying ROS, resulting in antioxidant defense capacity [78]. Drought ameliorates active accumulation of solutes (e.g., proline, α-tocopherol and polyphenol) to protect them against oxidative damage and allows plants to maintain positive turgor pressure, a requirement for maintaining stomata aperture and gas exchange [79]. Besides, non-enzymatic antioxidants like, leaf pigments, ascorbic acid, carotenoids, phenolics and flavonoids have a protective role to avoid ROS generation [80].

Thus, there are three general types of response to drought stress including [81]: a) mechanisms to avoid water loss (*e.g.* osmotic adjustment), b) mechanisms for protection of cellular components (*e.g.* qualitative and quantitative changes of pigments), and c) mechanisms of repairing against oxidative damage (*e.g.* antioxidant systems).

Excessive accumulation of reactive oxygen species (hydrogen peroxide, H_2O_2; superoxide, $O_2^{\bullet-}$; hydroxyl radical, OH• and singlet oxygen, $^1O_2$), and malondialdehyde are enhanced under abiotic and/or biotic stresses, which can cause oxidative damage to plant macromolecules and cell structures, leading to inhibition of plant growth and development, or to death. Among the various ROS, freely diffusible and relatively long-lived H_2O_2 acts as a central player in stress signal transduction pathways. These pathways can then activate multiple acclamatory responses that reinforce resistance to various abiotic and biotic stressors. To utilize H_2O_2 as a signaling molecule, non-toxic levels must be maintained in a delicate balancing act between H_2O_2 production and scavenging.

1.2.3 Drought effects on antioxidant enzymes

Drought stress causes oxidative stress by decreasing stomatal conductivity that confines CO_2 influx in to the leaves, reduces the leaf internal CO_2, leads to the formation of ROS such as hydroxyl radicals (OH•) singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), alkoxyl radical (RO) and superoxide radical (O_2•-) by enhancing electrons leakage to oxygen molecule [82-85]. In plant cell, mitochondria, chloroplasts and peroxisomes are the main location of ROS generation [86]. In addition, Environmental stress stimulates xanthine oxidase in peroxisomes, amine oxidase in the apoplast and NADPH oxidases (NOX) in the plasma membrane and produce ROS [87, 88]. Environmental stress induces excess ROS that can injure plant cells by oxidation of cellular components such as proteins, inactivate metabolic enzymes, DNA and lipids [89, 90].

The response to plant defense system to stress varies with the times, duration of contact and stress severity, type of organ or tissue and developmental stage [91, 92]. At a certain level, ROS works as an indicator molecule for activating acclimatory/protection responses through transduction pathways, where H_2O_2 acts as a secondary messenger [93, 94]. However, additional ROS induces harmful effects on plant cells. As a result, defenses against ROS are activated [95] by an array of nonenzymatic antioxidants [metabolites such as ascorbate (AsA), carotenoids, glutathione (GSH) and proline] and antioxidant enzymes [such as guaiacol peroxidases (GPOX), catalase (CAT), superoxide dismutase (SOD) and AsA-GSH cycle enzymes like glutathione reductase (GR) ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR)], work together for detoxification of ROS [88-89, 96-101]. In glutathione-ascorbate cycle, reduced glutathione is produced from oxidized glutathione through the donated electrons of all nonenzymatic and enzymatic antioxidants [89]. In addition to their damaging effects on cells, ROS can also take part as signaling molecules in many biological processes such as growth, enclosure of stomata, stress signaling and development [90, [102-104]. Recently more attention has been given to understand the antioxidant defense mechanism in plants exposed to drought stress [105-107]. Abiotic stress enhances the production of AsA–GSH and AsA–GSH cycle enzymes activities for cellular protection. Plant water relations play a significant role in the stimulation and/or modulation of antioxidative defense mechanism at drought stress [97-110].

1.2.4 Effect of salinity stress on nutrients, color parameters, leaf pigmentation, antioxidant phytochemicals, phenolic acid, flavonoid and antioxidant activity

Salinity is one of the major abiotic stressors which limits crop production and poses a serious threat to global food security. It prohibits the cultivation of vegetables in many areas in the globe. Approximately, 20% percent of the arable land and 50% of total irrigated land have varying levels of salinity [111]. Salinity stress induces a multitude of adverse effects on plants including morphological, physiological, biochemical, and molecular changes. It affects plant growth and development by creating osmotic stress by reducing the soil water potential and water uptake, causing specific ions (Na^+ and Cl^-) toxicity, stomatal closure, and reducing rate of photosynthesis [112].

All these physiological changes in plant aggravate overproduction of reactive oxygen species (ROS) that interferes normal cellular metabolism and results in oxidative damage by oxidizing proteins, lipids and DNA and other cellular macromolecules [88]. To counterbalance the osmotic stress, plants show variable adaptation processes such as enclosure of stomata,

metabolic adjustment, toxic ion homeostasis, and osmotic adjustment [112]. Plants have an excellent network of ROS detoxification system including, either non-enzymatic through protein, proline, carbohydrate, ascorbic acid (AsA), beta-carotene and carotenoids, phenolic compounds and flavonoids or through enzymatic antioxidants, such as superoxide dismutase (SOD), peroxidase (GPOX), catalase (CAT), and AsA peroxidase (APX) [88]. Salinity tolerance mechanisms in plants are remarkably varied among the species or even in different accessions of a species.

The leafy vegetables, *A. tricolor* comprises an excellent source of proximate and minerals, antioxidant leaf pigments, carotenoids, vitamins, phenolics and flavonoids. Natural antioxidants like leaf pigments, carotenoids, vitamins, phenolics and flavonoids have proven for health benefits as they detoxify ROS in the human body [6, 35]. These natural antioxidants play an important role in the human diet and involved in defense against several diseases like cancer, atherosclerosis, arthritis, cataracts, emphysema, and retinopathy, neuro-degenerative and cardiovascular diseases [8, 48, 50, 51]. *A. tricolor* is a popular leafy vegetable in many tropical and subtropical countries which is rich in nutrients, beta-carotene, vitamin C, polyphenols, flavonoids and antioxidants.

Compared to lettuce, *Amaranthus* contains 18 times more vitamin A, 13 times more vitamin C, 20 times more calcium and 7 times more iron. Amaranthus leaves contain 3 times more vitamin C, 3 times more calcium and 3 times more niacin than spinach leaves. [113]. It has been rated equal or superior in taste to spinach and is considerably higher in carotenoids (90-200 mg kg^{-1}), protein (14-30% on dry weight basis) and ascorbic acid (about 28 mg $100g^{-1}$) [7]. Minerals are of critical importance in the diet, even though they comprise only 4–6% of the human body. Major minerals are those required in amounts greater than 100 mg per day and they represent 1% or less of body weight. These include calcium, phosphorus, magnesium, sulfur, potassium, chloride, and sodium. Trace minerals are essential in much smaller amounts, less than 100 mg per day, and make up less than 0.01% of body weight. Essential trace elements are zinc, iron, silicon, manganese, copper, fluoride, iodine, and chromium. The major minerals serve as structural components of tissues and function in cellular and basal metabolism and water and acid–base balance [114, 115].

Amaranth is a salt tolerant plant [116]. Salinity stress enhances the contents of these natural antioxidants in plants [117-119]. Therefore, salt-stressed plants could economically be the potential sources of antioxidants in human lifestyle. The natural antioxidants in diet play an important role in human health as they are involved in defense against several diseases such as cancer, atherosclerosis, arthritis, cataracts, emphysema, retinopathy, neuro-degenerative and

cardiovascular diseases [8, 48, 50, 51]. *A. tricolor* is a well acclimatized leafy popular vegetable to different biotic and abiotic stresses [70]. Various factors such as biological, environmental, biochemical, physiological, ecological and evolutionary processes, and salinity are involved in the quantitative and qualitative improvement of natural antioxidants in this vegetable crop [72]. Scant information is available on the effects of soil salinity stress on proximate and minerals, antioxidant leaf pigments, carotenoids, vitamins, phenolics and flavonoids in leafy vegetables like *A. tricolor*. However, salt stress elevated protein, ascorbic acid, phenolics, flavonoids and antioxidant activity and reduced the fat, carbohydrate, sugar, and chlorophyll pigments in *Cichorium spinosum* [117]. Alam *et al*. [118] observed that in purslane, different doses of salt concentrations increased total polyphenol content (TPC); total flavonoid content (TFC); and FRAP activity by 8–35%, 35%, and 18–35%, respectively. Similarly, in buckwheat sprouts, salinity stress remarkably increased phenolic compounds and carotenoids compared to non-saline condition [119].

1.3 Aim of The Study

The leafy vegetables, *A. tricolor* comprises an excellent source of proximate and minerals, antioxidant leaf pigments, carotenoids, vitamins, phenolics and flavonoids. Natural antioxidants like leaf pigments, carotenoids, vitamins, phenolics and flavonoids have proven for health benefits as they detoxify ROS in the human body. The natural antioxidants are involved in defense against several diseases such as cancer, atherosclerosis, arthritis, cataracts, emphysema, retinopathy, neuro-degenerative and cardiovascular diseases. It is a popular leafy vegetable in the South East Asia and is becoming increasingly popular in the rest of the continent and elsewhere due to its attractive leaf color, taste and nutritional value. A lot of variations in this vegetable germplasm have been observed in Bangladesh. But no efforts had not been taken to know the status of these functional phytochemicals in this vegetable in terms of genetic diversity as well as abiotic stress response in the globe. Therefore, the present investigations of this doctoral dissertation were undertaken to study the genetic diversity and effects of abiotic stress response of this vegetable in relation to proximate and minerals, antioxidant leaf pigments, carotenoids, vitamins, phenolics and flavonoids with following purposes.

- To estimate quality, vitamins, minerals, polyphenol, flavonoids, antioxidant capacity, antioxidant leaf pigments, foliage and biological yield and their variability in vegetable amaranth

- To determine contribution of the component traits towards yield potential
- To find out possible ways for improving quality, vitamins, minerals, polyphenol, flavonoids, antioxidant leaf pigments and antioxidant capacity without compromising foliage yield
- To find out appropriate selection parameters for the improvement of vegetable amaranth.
- To categorize vegetable amaranth genotypes based on the contribution of antioxidant, nutrient content, and contributing agronomic traits towards divergence and to identify genotype for utilization in future breeding program.
- To study the selected *A. tricolor* genotypes in response to drought and salinity stress in terms of proximate, minerals, antioxidant leaf pigments, carotenoids, vitamins, phenolics, flavonoids and antioxidant activity.
- To elucidate key growth, anatomical, physiological, non-antioxidative and antioxidative defense mechanisms involved in drought tolerant by comparing selected *A. tricolor* genotypes
- To elucidate key physiological, enzymatic and non-enzymatic pathways involved in ROS detoxification and tolerance of *A. tricolor* under drought stress.

CHAPTER 2

GENETIC DIVERSITY

2.1 Morphological and Nutritional Traits

Vegetable amaranth contains high amount of protein, dietary fiber, dietary minerals and antioxidant compounds like ascorbic acid, beta-carotene and minerals (Fe, Mn and Zn) [6, 19, 120-124]. It has high adaptability under varied soil and agro-climatic conditions and great amount of genetic variability and phenotypic plasticity [17, 18]. However, very little attention has been paid for genetic improvement of this underutilized crop plant. Improvement of foliage yield of vegetable amaranth with yield related morphological traits, protein, dietary fiber, dietary minerals and antioxidant compounds like ascorbic acid, beta-carotene and minerals (Fe, Mn and Zn) through the knowledge of variability, association, along with direct and indirect influence of these component traits on yield has so far been lacking.

2.1.1 Genetic variability for nutrient, antioxidant, yield and yield contributing morphological traits in vegetable amaranth.

Purpose of the study

Underutilized crops like chenopods, buckwheat, and amaranth have recently gained worldwide attention in this respect as these contain abundant amounts of all the common antioxidant vitamin and nutrients required for normal human growth. Amaranth contains minerals, beta carotenoid, ascorbic acid, protein with nutritionally critical amino acids *viz*. lysine and methionine in addition to dietary fiber [6, 13, 14, 121, 124]. Besides its immense nutritional importance, it can grow successfully under varied soil and agro-climatic conditions [17, 18]. Simultaneously, these crops do not require large inputs and can be grown in agriculturally marginal lands [125]. With the increase in the world's population demands increased production of food crops that should also be nutritionally superior to the existing ones. FAO statistics reveal that there is a high frequency of low birth weight children in the developing countries, which is primarily due to deficiency of micronutrients in the mother's diet.

In Bangladesh, there are lots of variations in vegetable amaranth germplasm. As a potential underutilized crop, vegetable amaranth has drowned attention to carry out extensive research efforts to ascertain its antioxidant vitamin and nutritional composition. The literature on for nutrient, protein, dietary fiber antioxidant vitamins and mineral, yield and yield contributing morphological traits of leaves is rare. Also, there is absolutely no information on

the qualitative improvement of foliage with special reference to nutrient, protein, dietary fiber antioxidant vitamins and mineral, yield and yield contributing morphological traits. To fill this knowledge gap, the objectives of the present investigation were to (i) estimate nutrient, protein, dietary fiber antioxidant vitamins and mineral, yield and yield contributing morphological traits in genotypes of vegetable amaranth available in Bangladesh, and (ii) to find out possible ways for improvement of nutrient, protein, dietary fiber antioxidant vitamins and mineral, yield contributing morphological traits without compromising foliage yield.

Materials and methods

Plant materials, site and cultural practices

The germplasm accessions of the vegetable amaranth (*Amaranthus tricolor*) collected from different eco-geographical regions of Bangladesh were used in this investigation. Forty- seven distinct and promising genotypes of vegetable amaranth were grown under two sub experiments in 2011, 2012 and 2013 with repetition for two years for each sub experiments in a randomized block design with three replications at the experimental field of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. Weeding and hoeing was done at 7 days interval. Irrigation was provided at 5-7 days interval. For foliage yield plants were cut at the base of the stem (base of ground level). The plot size for each treatment was 2 m^2 for foliage yield and 1 m^2 for antioxidant, quality and morphological traits for sub experiment1, 4 m^2 for foliage yield and 1 m^2 for vitamin and mineral composition measurement for sub experiment2 and 4 m^2 for foliage yield and 1 m^2 for nutrient and antioxidant and yield contributing morphological traits for sub experiment3. Spacing was maintained with row-to-row and plant-to-plant distance 20 cm and 5 cm, respectively for sub expeiment1 and 25 cm and 5 cm from row-to-row and plant-to-plant, respectively were maintained for sub expeiment2 and 3. Recommended fertilizer and compost doses, appropriate cultural practices were maintained.

Data collection on plant traits

Data were collected at 30 days after sowing of the seeds for both the years for two sub experiments. The data were recorded from 10 randomly selected plants from each replication for plant height (cm), leaves $plant^{-1}$ and stem base diameter (cm). Foliage yield were harvested on whole plot basis. Beside this, five antioxidant traits viz., beta-carotene (mg g^{-1}), ascorbic acid (mg 100 g^{-1}) and iron (mg kg^{-1}), zinc (mg kg^{-1}) and Mn (mg kg^{-1}) and protein (mg 100 g^{-1}), fiber (%) and Ca (g 100 g^{-1}), K (g 100g^{-1}), Mg (g 100g^{-1}) were estimated.

Extraction and estimation of antioxidant vitamin

Beta Carotene

The extraction and estimation of carotenoid was done following the protocol previously described by Jensen [126]. To carry out the extraction process, 500 mg of fresh leaf sample was grinded in 10 ml of 80% acetone and centrifuged at 10,000 rpm for 3–4 min. The supernatant was taken and volume was made up to 20 ml in a volumetric flask. The absorbance values were taken at 510 nm and 480 nm.

The beta carotene was calculated by the following formula:

Amount of beta carotene = 7.6(Abs.at 480) - 1.49(Abs.at 510) ×Final volume/ (1000 × fresh weight of leaf taken).

Ascorbic acid

Ascorbic acid was analyzed by the method given by Glick [127]. To extract the sample, 5 gm fresh leaves were grinded with 5% H_3PO_3 – 10% acetic acid (5% Meta phosphoric acid (H_3PO_3) –10% acetic acid was prepared by dissolving 50 gm of H_3PO_3 in 800 ml of distilled water + 100 ml of glacial acetic acid and volume was made up to 1 liter with distilled water) for 1–3 min. The amount of extracting fluid was taken such that it should yield 1–10 µg of ascorbic acid/ml. In the solution, 1–2 drops of bromine was added and stirred until the solution became yellow. The excess bromine was decanted into bubbler and air was passed till bromine color disappeared. The bromine oxidized solution was placed in 2 matched tubes. In first tube 1 ml of 2, 4-DNP thio urea reagent (2,4-dinitrophenyl hydrazine-thio urea reagent was prepared by dissolving 2 gm 2,4-DNP in 100 ml of 9 N H_2SO_4. Four gm thio urea was added and dissolved in this solution. The filtered solution was added and the tube was placed in water at 37◦ C for 3 h. 5 ml of 85% H_2SO_4 (100 ml distilled water +900 ml conc. H_2SO_4; sp.gr. 1.84) was added drop wise by the burette in the tube, placed in a beaker of ice water. In second tube, 1 ml of 2, 4-DNP thio urea reagent was only added to prepare blank solution. After 30 min, the absorbance reading of the sample was taken at the wavelength of 540 nm by spectrophotometer. The blank solution was used for setting the zero transmittance of the spectrophotometer. The standard solution was prepared by dissolving 100 mg ascorbic acid of highest purity in 100 ml of 5% H_3PO_3–10% acetic acid. The solution was oxidized with bromine water as above. 10 ml of this dehydrated ascorbic acid was pipette in 500 ml volumetric flask and the solution was made up to 500 ml with the 85% H_2SO_4 solution. The solutions of different dilution were prepared by pipetting 5, 10, 20, 30, 40, 50 and 60 ml of the above solution into 100 ml volumetric flasks and volumes were made up to 100 ml of each by addition of 85% H_2SO_4 ml solution of each flask was taken separately and further the procedure was followed as discussed

above for the sample. The calibration curve was prepared by plotting absorbance values against concentration of ascorbic acid (in µg).

The amount of ascorbic acid (mg/100 gm) was calculated as follows:

Ascorbic acid content (mg/100 gm) = (µg from curve)/1000 × (ml of extract taken)/4 × 100/(sample wt. in gm)

Extraction and estimation of fiber

Fiber content was estimated using the method proposed by Watson [128]. The 500 mg dried leaves sample was extracted by boiling for 30 min in 50 ml of 5% H_2SO_4 and 75 ml of distilled water. The sample was filtered through linen cloth after 1 h with the addition of some cold distilled water and residue was washed twice with distilled water. In the residue, 50 ml of 5% KOH was added and volume was made up to the original volume. Further, the solution was boiled for 30 min and allowed to stand for some time after adding little cold distilled water and filtered through linen cloth. The residue was again washed with hot distilled water followed by a mixture of dilute HCl (HCl:H_2O in ratio of 1:2) and 5 ml ethyl alcohol. The residue was finally dried in a crucible at 80–100 °C and dried weight was measured and represented as percentage of initial material taken.

Extraction and estimation of protein

Protein was estimated following the method of Lowry *et al.* [129]. Briefly, 500 mg fresh vegetable amaranth leaves were washed and grinded in 1 ml of 20% trichloro acetic acid and placed over night. Next day supernatant was discarded and the residue washed thoroughly 2 – 3 times with distilled water. The chlorophyll was removed from the residue by adding sufficient amount of 80% acetone solution and centrifugation. After the removal of chlorophyll, the sample was dried in vacuum to evaporate the acetone. The pellet was digested with 1 ml of 0.5 N NaOH at 80 °C for 10 min in water bath. Further, 4 ml of distilled water was added and the sample was centrifuged at 7500 rpm. An aliquot of 0.5 ml was taken and 5 ml B.C. reagent (The B.C. reagent was prepared by adding 50 mg $CuSO_4.5H_2O$ in 10 ml of 2% sodium tartrate and 1 ml of this solution was added to 50 ml of 2% sodium carbonate prepared in 0.1 N NaOH) was added. After 10 min the color was developed by the addition of 0.5 ml 1 N Folin-Ciocalteu's reagent in the sample. The absorbance values were taken at wavelength of 640 nm on spectrophotometer. The standard graph was plotted against concentration of protein and absorbance values, using bovine albumen serum protein of 0.2, 0.4, 0.6, 0.8 and 1 µg/ml concentrations. The amount of protein in the sample can be calculated by comparing (interpolation) with the standard graph and expressed as mg/100 mg of fresh sample weight taken initially.

For determination of mineral nutrient and antioxidant mineral composition, the leaves were first oven dried and then digested in a 1:4 mixture of $HClO_3$ and HNO_3. Calcium was determined by flame photometry and iron, zinc and manganese were determined using atomic absorption spectrophotometer (Perkin Elmer 5100) [130, 131]

Statistical analysis

The raw data of consecutive two years for each sub experiments were compiled by taking the means of all the plants taken for each treatment and replication for different traits. The mean data of consecutive two years were averaged and the averages of two years means were statistically and biometrically analyzed. Analysis of variance was done according to Panse and Sukhatme [132] for each character. Genotypic and phenotypic variances, genotypic (GCV) and phenotypic (PCV) coefficient of variations, heritability (h^2_b) in broad sense, and genetic advance (GA%) were estimated according to Singh and Chaudhary [133]. Correlation coefficient was analyzed following Johnson *et al*. [134]. Path coefficient analysis was calculated according to the formula given by Dewey and Lu [28].

Results and discussion

Anemia, night blindness, scurvy, is the problem for poor child community in the third world countries including Indian subcontinent. Iron, beta carotene and ascorbic acid are also important for recovery of anemia, night blindness and scurvy, respectively. Antioxidant vitamins and minerals are important constituents of the human diet by serving as cofactors for many physiological and metabolic processes.

The analysis of variance revealed significant differences among the genotypes for all the all traits, which was the indication of the validity of further statistical analysis due to the presence of a wide range of variability among the 47 genotypes of vegetable amaranth (Table 1). Mean performance, %CV and CD for antioxidant and nutrient content, number of leaves per plant and foliage yield in 47 vegetable amaranth genotypes are presented in Table 1.

Variability Studies

Variability plays a vital role in the selection of superior genotypes in crop improvement program. Pronounced variation in the breeding materials is a prerequisite for development of varieties to fulfill the existing demand. Economically important traits are generally quantitative in nature that interacts with the environment where it is grown. This is why; breeder should calculate the variability by partitioning into genotypic, phenotypic, and environmental effects. Creation of variability is prerequisite for crop breeders. morphological traits are quantitative in nature, and interact with the environment under study, so partitioning the traits into genotypic,

phenotypic, and environmental effects is essential to find out the additive or heritable portion of variability. The mean, range, genotypic and phenotypic variance (Vg, Vp and coefficient of variation (GCV, PCV), h^2b, GA and GA in percent of mean are presented in Table 1. In the present investigation, the range of variation was much pronounced for all the traits except Ca, Mg, K, protein and beta-carotene content indicating a wide range of variability among the genotypes studied. High genotypic and phenotypic variances were observed for Fe, Zn, Mn, ascorbic acid, plant height, fiber content, and leaves per plant indicating the presence of the wide range of variability among the traits in vegetable amaranth.

Table 1. Genetic parameters for nutrient, antioxidant, yield and yield contributing morphological traits in vegetable amaranth

Character	Mean	Range	V_p	V_g	PCV	GCV	h^2_b (%)	GA (5%)	%GAPM
Ca (g/100 g)	1.70	0.76-2.15	0.18	0.16	24.96	23.53	88.89	0.87	51.41
Mg (g/100 g^1)	2.85	2.32-3.10	0.03	0.02	5.86	5.08	75.29	0.26	9.09
K (g/100 g)	3.98	1.60-6.65	2.50	2.35	39.73	38.52	94.00	3.26	81.84
Fe (mg kg^{-1})	1188.69	632.27-2324.94	161439.68	161325.15	33.80	33.79	99.93	827.11	69.58
Zn (mg kg^{-1})	818.68	449.68-1235.01	38087.71	37882.21	23.84	23.77	99.46	399.86	48.84
Mn (mg kg^{-1})	113.18	62.70-155.68	713.07	687.98	23.59	23.17	96.48	53.07	46.89
Protein (mg/100 g)	1.25	1.06-1.51	0.17	0.13	32.98	28.84	76.47	0.85	67.95
Fiber (%)	8.17	6.64-9.76	0.73	0.65	10.46	9.87	89.04	1.76	21.54
Beta carotenoid (mg/g)	0.85	0.60-1.15	0.22	0.19	55.18	51.28	86.36	0.97	113.67
Ascorbic acid (mg/100 g)	115.00	65.50-178.55	999.50	995.75	27.49	27.44	99.62	65.13	56.63
Plant height (cm)	21.77	9.50-40.72	53.90	53.55	33.72	33.61	99.35	15.12	69.47
Leaves/plant	9.75	4.92-22.25	16.15	16.12	41.22	41.18	99.81	8.28	84.91
Stem base diameter (cm)	6.41	2.6-12.54	5.61	5.56	36.95	36.79	99.11	4.88	76.12
Foliage yield/plot (kg)	4.57	3.75-5.95	5.79	5.65	52.65	52.01	97.58	4.96	108.47

Vp = Phenotypic variance, Vg = Genotypic variance, PCV = Phenotypic co-efficient of variation, GCV = Genotypic co-efficient of variation, h^2_b = heritability in broad sense, GA = Genetic advance, GAPM = Genetic advance in per-cent of mean, Fe = Iron, Zn= Zinc, Mn = manganese, Mg= magnesium, K= potassium.

In contrast, Ca, Mg, K, protein and beta-carotene content showed low genotypic and phenotypic variances that indicated no scope of selection on the basis of these traits for improvement of vegetable amaranth crop. Fe, Zn, Mn, ascorbic acid, plant height, fiber content, leaves per plant and foliage yield had close differences in genotypic and phenotypic variances along with genotypic coefficient of variability (GCV) and phenotypic coefficient of variability (PCV) values, which indicated preponderance of additive gene effects for these traits i. e., less environmental influence in the expression of these traits or the major portion of the phenotypic variance was genetic in nature and greater scope of improvement of vegetable amaranth through selection. Variability alone is not of much help in determining the heritable portion of variation. The amount of gain expected from a selection depends on heritability and genetic advance in a trait. Heritability has been widely used to assess the degree to which a character may be transmitted from parent to offspring. Knowledge of heritability of a character is important as it indicates the possibility and extent to which improvement is possible through selection [135]. However, high heritability alone is not enough to make sufficient improvement through selection generally in advance generations unless accompanied by a substantial amount

of genetic advance [136]. The expected genetic advance is a function of selection intensity, phenotypic variance, and heritability and measures the differences between the mean genotypic values of the original population from which the progeny is selected. It has been emphasized that genetic gain should be considered along with heritability in coherent selection breeding program [19]. It is considered that if a trait is governed by non-additive gene action it may give high heritability but low genetic advance, which limits the scope for improvement through selection, whereas if it is governed by additive gene action, heritability and genetic advance would be high, consequently substantial gain can be achieved through selection. I these studies, the heritability was high for all the traits except beta carotene indicated the preponderance of additive gene action for these traits. High heritability coupled with high GA in percent of mean was observed for all the traits except Mg indicated that were govern to a great extent by additive gene. So, selection based on these traits would be effective for the improvement of vegetable amaranth.

Correlation Studies

The phenotypic and genotypic correlations between the various characters are presented in Table 2. The genotypic correlation analysis presented in Table 2 showed some interesting results. In the present investigation, the genotypic correlation coefficients were very much close to the corresponding phenotypic values for all the traits indicating additive type of gene action i.e., less environmental influence on the expression of the traits. The higher magnitude of genotypic correlation than respective phenotypic correlations between various characters in amaranth have also been reported by Shukla *et al*. [23] and Shukla and Singh [18]. From Table 2 it was revealed that foliage yield had a significant positive correlation with iron, manganese, protein, fiber content, ascorbic acid, plant height, leaves per plant and stem base diameter indicating selection for high iron, manganese, protein, fiber, ascorbic acid content and tall and thick plant with more leaves were closely associated with high foliage yield i.e., increase in with iron, manganese, protein, fiber content, ascorbic acid, plant height, leaves per plant and stem base diameter could lead to increase the foliage yield of vegetable amaranth genotypes. Shukla *et al.* [23] observed positive association of foliage yield with beta-carotene and ascorbic acid, plant height, diameter of stem base and fiber content [23].

Table 2. Genotypic and phenotypic correlation co-efficient (r_g and r_p) for nutrient, antioxidant, yield and yield contributing morphological traits in vegetable amaranth

Traits		Mg (g/ 100 g)	K (g/ 100 g)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Protein (mg/100 g)	Fiber (%)	Beta carotene (mg/g)	Ascorbic acid (mg/100 g)	Plant height (cm)	Leaves /plant	Stem base diameter (cm)	Foliage yield/plot (kg)
Ca (g/100 g)	r_g	-0.08	-0.015	0.152	0.305*	0.155	-0.432*	-0.012	0.121	-0.139	-0.327*	-0.400**	-0.555**	-0.141
	r_p	-0.08	-0.012	0.150	0.307*	0.154	-0.431*	-0.012	0.121	-0.137	-0.326*	-0.398**	-0.554**	-0.140
Mg (g/100 g)	r_g		-0.032	-0.088	0.060	0.206	-0.06	-0.067	0.045	0.020	-0.234	-0.075	-0.23	0.130
	r_p		-0.033	-0.089	0.060	0.204	-0.06	-0.066	0.045	0.021	-0.133	-0.075	-0.23	0.133
K (g/100 g)	r_g			-.009	0.074	-0.070	0.241	0.008	0.120	0.114	0.172	0.309	0.162	0.232
	r_p			-0.09	0.073	-0.069	0.240	0.007	0.119	0.112	0.170	0.308	0.160	0.230
Fe (mg kg^{-1})	r_g				0.177	0.112	0.112	0.018	0.135	0.292	-0.175	-0.052	-0.035	0.318*
	r_p				0.176	0.110	0.110	0.017	0.132	0.291	-0.172	-0.051	-0.035	0.317*
Zn (mg kg^{-1})	r_g					0.278	0.133	0.175	0.126	0.122	-0.335*	-0.257	-0.199	0.096
	r_p					0.277	0.130	0.174	0.125	0.120	-0.334*	-0.256	-0.198	0.095
Mn (mg kg^{-1})	r_g						-0.165	0.195	0.187	0.131	-0.395*	-0.128	-0.195	0.319*
	r_p						-0.164	0.194	0.185	0.129	-0.393*	-0.127	-0.194	0.318*
Protein (mg/100 g)	r_g							0.027	-0.218	0.173	-0.275	0.181	0.122	0.456**
	r_p							0.025	-0.217	0.172	-0.273	0.180	0.120	0.453**
Fiber (%)	r_g								-0.057	0.013	-0.119	0.158	-0.292	0.672**
	r_p								-0.055	0.012	-0.118	0.157	-0.291	0.670**
Beta carotene (mg/g)	r_g									0.069	0.375*	0.342*	0115	0.132
	r_p									0.067	0.372*	0.340*	0.114	0.130
Ascorbic acid (mg/100 g)	r_g										-0.378*	-0.118	0.140	0.338*
	r_p										-0.376*	-0.116	0.141	0.336*
Plant height (cm)	r_g											0.564**	0.432*	0.504**
	r_p											0.563**	0.431*	0.502**
Leaves/plant	r_g												0.235	0.514**
	r_p												0.234	0.512**
Stem base diameter (cm)	r_g													0.520**
	r_p													0.519**

* significant at 5% ** significant at 1%, r_p = phenotypic correlation coefficient, r_g = genotypic correlation coefficient

Similarly, Sarker and Mian [137] observed significant positive association between yield and its contributing traits in rice. Plant height had significant exhibited significant positive association with leaves per plant and stem base diameter. A Similar trend was observed by earlier work in *A. tricolor* [23]. Rest of the nutrient, antioxidant, yield and yield contributing morphological traits in vegetable amaranth antioxidant vitamins and minerals traits showed insignificant association with foliage yield. It indicated that selection for high vitamins and mineral content might be possible without compromising yield loss i. e., concomitant selection for high antioxidant and yield contributing traits lead to develop high foliage yielding vegetable amaranth varieties.

Considering high genotypic and phenotypic variances along with genotypic coefficient of variability and phenotypic coefficient of variability values, high heritability coupled with high genetic advance and genetic advance in percent of mean, six traits viz., Fe, Mn, Zn, protein, fiber, beta-carotene, ascorbic acid, plant height, leaves per plant, stem base diameter and foliage yield would be selected for the improvement of vegetable amaranth genotypes under study. However, correlation study revealed that strong positive association of Fe, Mn, protein, fiber, beta-carotene, ascorbic acid, plant height, leaves per plant and stem base diameter with foliage yield. Selection based on Fe, Mn, protein, fiber, beta-carotene, ascorbic acid, plant height, leaves per plant and stem base diameter could lead to increase the foliage yield of vegetable amaranth strains.

Path coefficient studies

Path coefficient analysis was carried out using genotypic correlation coefficient among fourteen nutrients, antioxidants, yield and its contributing traits to estimate the direct and indirect effect on foliage yield (Table 3). The fiber content, leaves plant^{-1} and plant height had high positive direct effect on foliage yield. High positive direct effect for fiber content, leaves plant^{-1} and plant height, moderate positive direct effect for stem base diameter Fe, Mn K and beta-carotene content in amaranth had been reported. On the other hand, high negative direct effect was observed in Ca content and negligible positive direct effect was found in Zn and protein content. Shukla *et al.* [23] also found similar results for protein content in same crop. The ascorbic acid showed negligible negative direct effect positive direct effect on foliage yield. It was interesting that path coefficient analysis results confirmed the similarity of the correlation coefficient analysis results. Calcium had high negative direct effect and insignificant negative correlation. Potassium had considerable positive direct effect and

Table 3. Partitioning of genotypic correlation into direct (bold phase) and indirect effect for nutrient, antioxidant, yield and yield contributing morphological traits in vegetable amaranth

Traits	Ca (g/ 100 g)	K (g/ 100 g)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Protein (mg/100 g)	Fiber (%)	Beta carotene (mg/g)	Ascorbic acid (mg/100 g)	Plant height (cm)	Leaves /plant	Stem base diameter (cm)	Genotypic correlation with foliage yield $plot^{-1}$ (kg)
Ca (g/100 g)	**-0.300**	-0.004	0.012	0.003	0.046	0.050	-0.003	0.004	0.005	0.103	-0.131	0.070	-0.141
K (g/100 g)	0.006	**0.230**	0.0001	0.0002	0.0001	0.002	0.002	0.043	0.020	-0.051	0.031	0.003	0.232
Fe (mg kg^{-1})	-0.019	-0.002	**0.290**	0.001	0.003	-0.007	0.009	0.021	-0.010	0.039	-0.020	0.011	0.318*
Zn (mg kg^{-1})	-0.094	-0.001	0.031	**0.083**	0.064	0.001	0.008	-0.035	-0.004	0.091	-0.074	0.025	0.096
Mn (mg kg^{-1})	-0.054	-0.016	0.003	0.002	**0.260**	0.004	-0.015	0.012	-0.005	0.127	-0.041	0.026	0.319*
Protein (mg/100 g)	0.168	0.002	0.168	0.055	-0.037	**0.058**	0.002	-0.075	0.028	0.079	0.008	0.002	0.456**
Fiber (%)	0.004	0.003	0.004	0.001	0.037	0.000	**0.621**	-0.034	0.000	0.029	0.016	-0.006	0.672**
Beta-carotene (mg/g)	-0.028	-0.001	0.028	-0.003	0.021	0.001	0.026	**0.141**	-0.022	-0.126	0.102	-0.008	0.132
Ascorbic acid (mg/100 g)	0.040	-0.002	0.152	0.001	0.032	0.000	0.024	0.008	**-0.038**	0.130	-0.006	-0.005	0.338*
Plant height (cm)	-0.179	0.001	0.123	0.103	0.102	-0.006	-0.137	-0.156	-0.107	**0.518**	0.181	0.062	0.504**
Leaves $plant^{-1}$	0.120	0.003	-0.012	-0.002	-0.032	-0.002	0.063	0.044	0.001	-0.175	**0.537**	-0.028	0.514**
Stem base diameter (cm)	-0.159	0.004	0.016	0.002	0.050	-0.004	0.033	-0.070	0.102	0.149	0.068	**0.333**	0.520**

insignificant positive correlation. Zn had negligible positive direct effect and insignificant positive correlation. Protein exhibited negligible positive direct effect and significant positive correlation. Direct selection based on these three nutrient traits (Ca, K, Zn and protein) would not be effective for the improvement of foliage yield of vegetable amaranth. Concomitant selection based on high nutrient content and high foliage yield would be effective for the improvement of vegetable amaranth. Manganese and Fe showed considerable positive direct effect with considerable positive genotypic correlation, so direct selection based on Fe and Mn would be effective for the improvement of vegetable amaranth. Beta-carotene exhibited moderate positive direct effect but its negative indirect effect via plant height made negligible genotypic correlation on foliage yield. Ascorbic acid had negligible negative direct effect with significant genotypic correlation on foliage yield. Direct selection based on antioxidant traits (beta-carotene and ascorbic acid) would not be effective for improving foliage yield. Rather, concomitant selection with high antioxidant and high foliage yield would be effective selection method for improvement of vegetable amaranth. Fiber content, leaves plant^{-1} and plant height had high positive direct effect and stem base diameter had moderate positive direct effect along with highly significant positive genotypic correlation with foliage yield. Shukla *et al.* [25] observed similar findings for plant height, fiber and beta-carotene content in vegetable amaranth. Direct selection on the basis of fiber content, leaves plant^{-1}, plant height and stem base diameter would significantly improve the foliage yield of vegetable amaranth. Selection based on plant height and leaves/plant concomitantly required considering Ca and beta-carotene content of the genotypes.

Considering all genetic parameters, Ca, Mg, K, protein and beta-carotene content all the traits studied would be selected for the improvement of 47 vegetable amaranth genotypes. However, correlation study revealed that selection based on Fe, Mn, protein, fiber, plant height, leaves/plant and stem base diameter could lead to increase the foliage yield of vegetable amaranth genotypes. Based on mean, range, genetic parameters, correlation coefficient values and path coefficient values finally we could conclude that direct selection through Fe, Mn, fiber, plant height, leaves/plant and stem base diameter would significantly improve the foliage yield of vegetable amaranth. Concomitant selection based on high nutrient and antioxidant content and high foliage yield would be effective for improvement of vegetable amaranth.

Lot of variability in respect of nutrient, antioxidant, yield and yield contributing morphological traits were observed among the germplasm while analyzing genetic parameters, correlation and path coefficient values and interpretation of these results. Breeder may utilize the present findings for developing high yielding varieties with high nutrient and antioxidant

content in future. Further investigation may be carried out to confirm the study in different locations of Bangladesh for their stability analysis. Association of nutrient and antioxidant and yield contributing traits revealed that breeder can improve the foliage yield without compromising high nutrient, antioxidant and yield related morphological traits.

Abstract

Four-seven vegetable amaranth genotypes were evaluated to investigate nutrient, antioxidant, yield and yield contributing morphological traits and its genetic variability in a RCBD with three replications at Bangabandhu Sheikh Mujibur Rahman Agricultural University in Bangladesh. Significant mean sum of square revealed a wide range of genotypic variability among traits. Vegetable amaranth was rich in iron, zinc, manganese, magnesium and potassium. High mean, high range of variability and high genotypic variance were observed for all the traits except Ca, Mg, K, protein and beta-carotene content. Considering genetic parameter all the traits except Ca, Mg, K, protein and beta-carotene content would be selected for the improvement of vegetable amaranth genotypes under study. However, correlation study revealed that selection based on Fe, Mn, protein, fiber, ascorbic acid and plant height, leaves per plant and stem base diameter could lead to increase the foliage yield of vegetable amaranth genotypes. Based on mean, range, genetic parameters, correlation coefficient values and path coefficient values finally we could conclude that direct selection through Fe, Mn, fiber, plant height, leaves/plant and stem base diameter would significantly improve the foliage yield of vegetable amaranth. Insignificant genotypic correlations between foliage yield with most of nutrient, antioxidant, yield and yield contributing morphological indicating that selection for high nutrient, antioxidant and yield contributing morphological traits might be possible without compromising yield loss.

2.2 Agronomic Traits, Leaf Pigmentation, Antioxidant Phytochemicals and Antioxidant Activity

Vegetable amaranth is one of the popular leafy vegetables in the South-East Asia and is becoming increasingly popular in the Asia and elsewhere due to its attractive leaf color, taste and nutritional value. Amaranth leaves are a rich and inexpensive source of dietary fiber, protein, vitamins and a wide range of minerals and natural leaf pigments, TAC, TFC and antioxidants [3, 4, 6]. The interest of consumers in the aesthetic, nutritional and safety aspects of food has increased the demand for natural pigments such as chlorophyll, betalain, and carotene. Betalain are water-soluble compounds found in a limited number of families of the plant order Caryophyllales like *Amaranthus* have a unique source of betalain and important free radical-scavenging activity [30, 31]. betacyanin are red to purple colored betalain and yellow colored betalain known as betaxanthin [32]. Similarly, carotene grouped into alpha-carotene, beta-carotene and xanthophyll. Antioxidant vitamins and minerals, phenolic compounds and flavonoids protect the body from harmful free radicals such as superoxide, hydroxyl, hypochlorite, hydrogen peroxide, lipid peroxides and nitric oxide that cause damage to cells and impair the immune system and lead to infections and various degenerative diseases like heart disease, neuro-degenerative disease, atherosclerosis, cancer, arthritis, cataracts, emphysema, retinopathy [49, 52].

2.2.1 Variability, heritability and genetic association in vegetable amaranth (*Amaranthus tricolor* L.)

Purpose of the study

Amaranth leaves are a rich and inexpensive source of dietary fiber, protein, vitamins and a wide range of minerals [3, 4, 6]. The species of *Amaranthus tricolor* L. grown as leafy vegetables are loosely termed as vegetable amaranth; it is a self-pollinated C4 crop with wide genetic diversity and phenotypic plasticity [142]. The species used as vegetable types have short plants with large smooth leaves, small auxiliary inflorescences, and succulent stems. In Bangladesh, we found lots of variations in vegetable amaranth germplasm in respect of antioxidant, yield and yield related traits [143]. Generally, the success of any crop improvement program largely depends on the magnitude of genetic variability, heritability, genetic advance, character association. Genetic variability is important for selection of parents with transgressive segregants [144]. Heritability estimates, provide information on the proportion of phenotypic variance that is due to genetic factors for different traits but heritability estimate alone is not a sufficient measure of the level of possible genetic progress. Effective selection can be made when the value broad sense heritability estimates is considered together with

selection differential or genetic advance [145]. Information on the amount and direction of association between yield and yield related characteristics is important for rapid progress in selection and genetic improvement of a crop [146]. Correlations between two or more plant characters and yield provide suitable means for indirect selection for yield. Extensive research efforts have been carried out to ascertain the mineral composition of vegetable amaranth. Although some reports on its nutritional aspects are available [4, 7, 147], there are few works on mineral composition of leaves along with qualitative improvement of foliage with special reference to leaf attributes [9, 148]. So, the present investigation was carried out (i) to estimate quality, biological yield and composition of minerals in 43 different cultivated genotypes of vegetable amaranth available in Bangladesh, and (ii) to find out possible ways for improvement of protein, dietary fiber, K, Ca and Mg compositions without compromising biological yield.

Material and methods

The experiment was conducted at the experimental field of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. The experimental site was located in the center of the Madhupur Tract (AEZ28), about 24°23´N 90°08´E, with a mean elevation of 8.4 m. s. l. The experimental field was a high land having silty clay soil. The soil was slightly acidic (pH 6.4) and low in organic matter (0.87%), total N (0.09%) and exchangeable K (0.13 cmol/kg). The site falls under the subtropical Zone and has mean temperatures of 29 °C (summer) and 18 °C (winter). Based on our previous studies, 43 genotypes were selected from102 genotypes based on our previous studies for further confirmation of that selected genotypes on agro-nutritional traits. The genotypes were locally well adapted and cultivated as varieties by local farmers. The genotypes were sown in a randomized complete block design (RCBD) with five replications, during three successive years (2013, 2014 and 2015). Each accession was sown in two-unit plots, one of 1 m^2 for the biological yield and other of 0.6 m^2 for the mineral, quality and agronomic traits study. The spacing was 20 cm from row-to-row and 5 cm from plant-to-plant, respectively. Recommended fertilizer dose, appropriate cultural practices were maintained. To record the data on biological yield, plants were cut at the base of the stem (base of ground-level). Data were collected at 30 days after seed sowing, on 10 randomly selected plants in each replication for four agronomic traits such as leaf area (cm), shoot weight (g), shoot/root weight and stem base diameter (cm). Biological yield was recorded on whole plot basis. Beside this, content percentages of three minerals, K, Ca and Mg and of protein and dietary fiber, were estimated.

Estimation of protein, dietary fiber, minerals

Protein, dietary fiber, minerals were measured following the procedure described in the previous chapter

Statistical analysis

The raw data of consecutive three years (2012-2014) were compiled by taking the means of all the plants taken for each treatment and replication for different traits. The mean data of consecutive three years were averaged and the averages of three years means were statistically and biometrically analyzed. Analysis of variance was done according to (Panse and Sukhatme [132] for each character. Genotypic and Phenotypic variances, Genotypic (GCV) and Phenotypic coefficient of variation (PCV), heritability (h^2_b) in broad sense and genetic advance in percent of mean (GAMP) were estimated according to Singh and Chaudhary [133]. Correlation coefficient was analyzed following Johnson *et al*. [134].

Results and discussion

Mean performance, coefficient of variation (CV%) and critical difference (CD) for mineral content, quality and agronomic traits and biological yield for 43 vegetable amaranth genotypes are presented in Table 1. The analysis of variance revealed significant differences among the genotypes for the ten traits studied, indicating the validity of further statistical analysis.

Mineral composition

Potassium

Accession VA6 had the highest K content (1.60%), followed by VA16 (1.24%) and VA1 (1.12%). The lowest amount of K was found in VA36 (0.84%). The mean K content was 1.014%. The estimated CV for K was the highest among all minerals (0.71%).

Calcium

The average Ca content was 2.476%. The highest amount of Ca was found in VA31 (3.47%) followed by VA1 (3.25%) and VA28 (3.18%), while the lowest amount was found in the leaves of VA9 (1.49%). The CV for Ca (0.51%) was less than for K. Twenty genotypes showed above-average mean values for Ca content.

Magnesium

The average Mg content was 2.984%. The highest Mg content was observed in VA6 (3.53%), followed by VA16 (3.24%), and VA1, VA5 and VA19 (3.10% the three of them), whereas the lowest Mg content was observed in VA24 (2.84%). The CV (0.22%) was the least among all the minerals analyzed. Out of 43 genotypes, 18 showed above-average values for Mg content.

Table 1. Mean performance, %CV and CD for mineral, quality and agronomic traits in 43 vegetable amaranth genotypes.

Genotype	K%	Ca%	Mg%	Protein %	Dietary fiber%	Leaf area (cm^2)	Shoot weight (g)	Shoot /root weight	Stem base diameter (cm)	Biological yield m^{-2} (g)
VA1	1.12	3.25	3.10	1.24	7.31	56.08	12.92	13.11	2.77	1163.57
VA2	1.08	2.78	2.97	1.19	8.81	48.05	14.51	15.09	4.17	1322.60
VA3	1.03	2.05	3.04	1.27	9.51	60.29	15.53	26.39	4.83	1386.81
VA4	1.09	2.69	2.89	1.07	8.85	134.74	18.63	12.08	5.06	1666.19
VA5	1.07	2.05	3.10	1.08	7.31	86.62	11.53	15.43	6.47	1067.05
VA6	1.60	2.22	3.53	1.03	7.35	155.08	16.78	10.19	5.04	1516.08
VA7	1.05	2.39	3.04	1.06	8.08	114.93	18.42	10.17	5.73	1658.88
VA8	1.00	2.62	2.97	1.12	7.82	140.79	21.12	13.58	7.99	1900.01
VA9	0.97	1.49	2.85	1.29	7.74	58.45	15.42	14.90	4.88	1387.92
VA10	0.94	1.59	3.04	1.22	8.51	217.78	21.27	26.24	9.74	1914.98
VA11	0.97	2.45	3.04	1.42	8.31	206.43	11.09	10.75	6.51	997.09
VA12	0.97	2.39	3.00	1.11	7.75	130.38	18.81	8.36	6.27	1697.58
VA13	0.99	1.65	2.85	1.18	9.09	272.54	23.59	13.19	10.79	2131.40
VA14	0.97	1.90	2.91	1.28	6.74	294.59	25.49	10.41	11.45	2295.29
VA15	0.98	1.90	2.97	1.15	7.43	222.82	21.56	12.26	6.98	1946.94
VA16	1.24	1.76	3.24	1.13	7.82	187.84	28.98	18.28	8.61	2628.43
VA17	0.97	2.29	3.00	1.03	9.33	102.83	12.22	10.63	5.83	1098.52
VA18	0.97	3.09	3.00	1.04	8.21	299.67	24.82	14.80	5.08	2238.83
VA19	0.98	2.70	3.10	1.47	9.75	33.89	18.72	13.45	6.09	1687.90
VA20	1.00	2.39	3.04	1.41	7.71	120.80	12.45	14.85	6.40	1121.35
VA21	1.00	3.02	3.07	1.30	7.91	71.34	13.46	15.36	2.99	1242.66
VA22	0.95	3.01	3.04	1.23	6.65	123.63	18.12	13.68	6.30	1631.26
VA23	1.00	2.14	2.91	1.06	8.21	139.31	13.58	30.69	3.25	1232.58
VA24	1.03	1.89	2.84	1.03	9.55	136.30	10.20	12.34	5.84	918.04
VA25	1.03	2.53	2.97	1.14	8.37	197.76	17.07	10.62	8.23	1577.22
VA26	1.02	2.29	2.85	1.49	5.97	131.17	26.33	70.29	4.14	2372.89
VA27	1.01	2.79	2.85	1.17	6.02	90.72	27.58	44.09	4.60	2485.66
VA28	0.97	3.18	3.04	1.59	6.98	150.86	14.09	4.26	6.36	1278.67
VA29	0.98	2.85	2.97	1.29	7.25	69.85	14.47	14.14	4.56	1373.83
VA30	0.96	2.53	2.91	1.08	8.25	110.41	16.02	10.58	5.81	1435.89
VA31	1.00	3.47	3.04	1.01	8.74	99.87	17.32	10.38	5.55	1561.74
VA32	1.00	3.09	2.94	1.88	6.95	220.42	11.57	9.69	2.68	1051.46
VA33	1.00	2.39	2.94	1.56	7.77	127.01	10.16	12.29	5.47	954.37
VA34	0.95	2.29	2.84	1.69	7.20	156.98	12.25	8.68	7.10	1154.05
VA35	0.96	2.62	2.91	1.41	6.51	119.26	11.33	9.47	6.61	1042.90
VA36	0.84	2.38	2.94	1.23	6.68	159.98	10.17	9.78	6.43	936.51
VA37	1.03	2.79	3.01	1.38	6.20	210.38	15.76	8.81	7.32	1436.01
VA38	0.97	2.47	2.97	1.33	8.51	178.92	13.22	8.53	6.95	1182.90
VA39	0.98	2.39	2.91	1.62	7.85	114.59	18.26	8.13	5.97	1664.55
VA40	1.00	2.62	2.91	1.36	9.15	234.54	13.14	9.88	6.98	1187.71
VA41	0.97	2.69	2.97	1.18	6.84	117.52	17.06	15.73	6.10	1552.88
VA42	0.98	2.45	2.91	1.13	7.64	60.67	13.82	12.96	4.84	1256.58
VA43	0.98	2.94	2.90	1.15	7.35	109.92	17.52	9.57	5.49	1566.22
Mean	1.014	2.476	2.984	1.258	7.81	141.30	16.66	14.98	6.05	1509.86
F-value	**	**	**	**	**	**	**	**	**	**
SE	0.417	0.734	0.382	0.606	0.727	0.214	0.605	0.409	0.537	2.205
CV%	0.71	0.51	0.22	0.84	0.16	3.24	1.16	1.57	0.98	5.28
CD	0.203	0.357	0.186	0.295	0.3542	0.1043	0.294	0.199	0.2614	10.74

K = Potassium, Ca = Calcium, Mg = magnesium, ** Significant in1% level

Quality traits

Protein content

The average protein content was 1.258%. VA32 showed the highest protein content (1.88%) followed by VA34 (1.69%), VA39 (1.62%), VA28 (1.59%) and VA33 (1.56%). On the other hand, the lowest protein content was observed in VA31(1.01%). The CV for protein (0.84%)

was the highest between the two quality traits analyzed. Out of 43 genotypes, 18 showed above-average values for protein content.

Dietary fiber content

The highest dietary fiber content was found in VA19 (9.75%), followed by VA24(9.55%), VA3 (9.51%), VA17 (9.33%) and VA40 (9.15%). In contrast, the lowest dietary fiber content was observed in VA26 (5.97%). The average dietary fiber content was 7.81%. The CV for dietary fiber (0.16%) was the lowest among all the quality traits analyzed. Out of 43 accessions, 21 showed above-average values.

Agronomic traits

Leaf area

The highest leaf area was found in VA18 (299.67 cm^2), followed by VA14 (294.59 cm), VA13 (272.54 cm^2) and VA40 (234.54 cm^2), whereas, the lowest leaf area was found in VA2 (48.05 cm^2). The average leaf area was 141.30 cm^2. The CV for leaf area was 3.24%. Out of 43 accessions, 16 showed above average values.

Shoot weight

The highest shoot weight was found in VA16 (28.98 g), followed by VA27 (27.58 g), VA26 (26.33 g), VA14 (25.49 g), VA18 (24.82 g) and VA13 (23.59 g). Conversely, the lowest shoot weight was observed in VA33 (10.16 g) followed by VA36 (10.17 g). The mean shoot weight was 16.66 g. The CV for shoot weight was 1.16%. Twenty accessions showed above-average values.

Shoot/root weight

The highest shoot/root weight was found in VA26 (70.29), and the lowest in VA39 (8.13), followed by VA12 (8.36) VA38 (8.53) VA34 (8.68) VA37 (8.81). The average was 14.98. The CV for shoot/root weight was 1.57%. Ten accessions showed above-average values.

Stem base diameter

The highest value was found in VA14 (11.45 cm), followed by VA13 (10.79 cm). The lowest value was observed in VA1 (2.77 cm), followed by VA32 (2.68 cm) and VA21 (2.99 cm). The average was 6.05 cm. The CV (0.98%) was the lowest among the agronomic traits analyzed. Twenty-one accessions showed above-average values.

Biological yield

The highest value was found in VA16 (2628.43 g/m^2) followed by VA27 (2458.66 g/m^2), VA26 (2372.89 g/m^2), VA14 (2295.29 g/m^2), VA18 (2238.83 g/m^2) and VA13 (2131.40 g/m^2). The lowest value was observed in VA24 (918.04 g/m^2) followed by VA36 (936.51 g/m^2), VA33 (954.37 g/m^2) and VA11 (997.09 g/m^2). The average was 1509.86 g/m^2. The CV

(5.26%) was the highest among all the agronomic traits analyzed. Twenty accessions showed above-average values.

Variability studies

The genotypic and phenotypic variances (σ^2_g, σ^2_p) and coefficients of variation (GCV, PCV), h^2_b and GAMP are presented in Table 2. The highest genotypic variance was for biological yield (194457.42), followed by leaf area (4326.36). Shoot/root weight, shoot weight and dietary fiber content exhibited moderate genotypic variances. On the other hand, the lowest genotypic variance was observed for K (0.012) followed by Mg (0.015), Ca (0.212) and protein (0.040) contents. The phenotypic variances for all the traits were slightly higher but close to the genotypic variances. GCV values ranged from 4.10% (Mg) to 73.56% (shoot/root weight). The PCV values showed similar trends as GCV values and ranged from 4.37% (Mg) to 74.75% (shoot/root weight). The heritability estimates were high for all the traits and ranged from 85.71% (K) to 99.99% (biological yield). The highest expected genetic advance was exhibited for shoot/root weight (149.10%) followed by leaf area (95.83%), stem base diameter (63.40%), shoot weight (61.01%), and biological yield (60.16%). Moderate GAMP was found in Ca (38.13%), protein content (32.78%), dietary fiber content (25.43%) and K (20.60%). Variability plays a vital role for the selection of superior genotypes in crop improvement programs. Agronomic traits are quantitative in nature, and interact with environment under study, so partitioning the traits into genotypic, phenotypic, and environmental effects is essential to find out the additive or heritable portion of variability. In the present investigation, biological yield, leaf area, shoot/root weight, shoot weight and dietary fiber content had high to moderate genotypic and phenotypic variances along with GCV and PCV values, which indicate scope for improvement in these traits through selection due to predominance of additive gene action for these traits. Variability alone is not of much help in determining the heritable portion of variation. The amount of gain expected from a selection depends on heritability and genetic advance in a trait. Heritability has been widely used to assess the degree to which a character may be transmitted from parent to offspring. Knowledge of heritability of a character is important as it indicates the possibility and extent to which improvement is possible through selection [135]. However, high heritability alone is not enough to make sufficient improvement through selection generally in advance generations unless accompanied by substantial amount of genetic advance [136]. The expected genetic advance is a function of selection intensity, phenotypic variance and heritability and measures the differences between the mean genotypic values of the original population from which the progeny is selected. It has been emphasized that genetic gain should be considered along with

heritability in coherent selection breeding program [7]. It is considered that if a trait is governed by nonadditive gene action it may give high heritability but low genetic advance, which limits the scope for improvement through selection, whereas if it is governed by additive gene action, heritability and genetic advance would be high, consequently substantial gain can be achieved through selection. In the present study the heritability and genetic advance values were high for all the traits, indicating preponderance of additive gene effects.

Table 2. Genetic parameter for mineral, quality and agronomic traits in 43 vegetable amaranth genotypes.

Genetic parameter	K%	Ca%	Mg%	Protein %	Dietary fiber%	Leaf area (cm^2)	Shoot weight (g)	Shoot /root weight	Stem base diameter (cm)	Biological yield m^{-2} (g)
σ^2g	0.012	0.212	0.015	0.040	0.936	4326.36	24.41	121.40	3.48	194457.42
σ^2p	0.014	0.214	0.017	0.044	0.940	4332.27	24.48	125.38	3.49	194468.25
GCV	10.80	18.60	4.10	16.29	12.38	46.55	29.66	73.56	30.82	29.21
PCV	11.67	18.68	4.37	16.67	12.42	46.58	29.70	74.75	30.87	29.21
h^2_b	85.71	99.07	88.24	95.45	99.36	99.86	99.71	96.83	99.71	99.99
GAMP	20.60	38.13	7.94	32.78	25.43	95.83	61.01	149.10	63.40	60.16

K = Potassium, Ca = Calcium, Mg = magnesium, σ^2g = Genotypic variance, σ^2p = Phenotypic variance, GCV = Genotypic coefficient of variation, PCV = phenotypic coefficient of variation, h^2_b = Heritability in broad sense, GAMP = Genetic advance in percent of mean.

Correlation studies

Table 3 shows the phenotypic and genotypic correlations among the characters studied. The r_g (genotypic correlation coefficients) were very much close to the corresponding phenotypic values for all the traits. The biological yield had significant positive correlation with leaf area (0.326), shoot weight (0.999), shoot/root weight (0.454) and stem base diameter (0.368). Stem base diameter had a significant positive association with leaf area (0.597) and shoot weight (0.365), whereas this trait showed significant negative association with Ca (–0.491). Shoot/root weight exhibited significant positive interrelationship with shoot weight (0.454). Significant positive association was observed between shoot weight and leaf area (0.326). Among mineral content quality and agronomic traits and biological yield, only K exhibited a significant positive association with Mg (0.753) protein showed a significant negative association with dietary fiber (–0.295); and Ca had a significant negative association with stem base diameter (–0.491). The rest of the interrelationships among mineral, quality and agronomic traits were insignificant. The genotypic correlation coefficients were very much close to the corresponding phenotypic values for all the traits indicating additive type of gene action for the expression of these traits. Insignificant genotypic correlation was observed among mineral, quality and agronomic traits and biological yield, except K *vs.* Mg (0.753), protein *vs.* dietary fiber (–0.295), and stem base diameter *vs.* Ca (–0.491). This indicates that selection for high mineral, protein

and dietary fiber content might be possible without compromising yield loss. On the other hand, most of the interrelationships among different agronomic traits were significant. Similar trend was observed by earlier works in *A. tricolor* [7, 143]. Biological yield had significant positive correlation with leaf area (0.326), shoot weight (0.999), shoot/root weight (0.454) and stem base diameter (0.368), indicating that biological yield of vegetable amaranth could be increased with the increase of leaf area, shoot weight, shoot/root weight and stem base diameter. Sarker *et al*. [143] observed that foliage yield was highly associated with plant height, leaf area, leaves/plant stem base diameter and dietary fiber content. Similarly, Shukla *et al*. [23] observed a positive association of foliage yield with beta carotene and ascorbic acid. Stem base diameter had a significant positive association with leaf area (0.597), and shoot weight (0.365), whereas these traits showed significant negative association with Ca (-0.491).

Table 3. Genotypic and phenotypic correlation co-efficient (r_g and r_p) for mineral, quality and agronomic traits in 43 vegetable amaranth genotypes.

Traits		Ca%	Mg%	Protein %	Dietary fiber%	Leaf area (cm^2)	Shoot weight (g)	Shoot/ root weight	Stem base diameter (cm)	Biological yield m^{-2} (g)
K%	r_g	-0.091	0.753**	-0.256	0.012	-0.038	0.154	0.019	-0.124	0.153
	r_p	-0.093	0.755**	-0.258	0.013	-0.039	0.156	0.020	-0.126	0.155
Ca%	r_g		0.063	0.256	-0.194	-0.217	-0.183	-0.168	-0.491**	-0.182
	r_p		0.065	0.158	-0.196	-0.219	-0.184	-0.169	-0.493**	-0.184
Mg%	r_g			-0.214	0.042	-0.055	0.036	-0.179	-0.038	0.036
	r_p			-0.215	0.046	-0.057	0.038	-0.180	-0.039	0.037
Protein%	r_g				-0.295*	0.067	-0.255	-0.010	-0.084	-0.246
	r_p				-0.297*	0.069	-0.257	-0.013	-0.086	-0.248
Dietary fiber%	r_g					-0.065	-0.152	-0.246	0.074	-0.163
	r_p					-0.067	-0.155	-0.248	0.075	-0.166
Leaf area (cm^2)	r_g						0.326*	-0.127	0.597**	0.326*
	r_p						0.328*	-0.129	0.599**	0.328*
Shoot weight (g)	r_g							0.454**	0.365**	0.999**
	r_p							0.456**	0.367**	0.999**
Shoot /root weight	r_g								-0.226	0.454**
	r_p								-0.228	0.456**
Stem base diameter (cm)	r_g									0.368**
	r_p									0.369**

K = Potassium, Ca = Calcium, Mg = magnesium, * Significant in 5% level, ** Significant in1% level

Shoot/root weight exhibited significant positive interrelationship with shoot weight (0.454) indicating that plant with thick stem contained less Ca, more leaves and shoot weight. Significant positive association was observed between shoot weight and leaf area (0.326). Considering high genotypic and phenotypic variances along with GCV and PCV values, high heritability coupled with GAMP, five traits (leaf area, shoot/root weight, shoot weight, dietary fiber content and biological yield) could be selected for the improvement of 43 vegetable amaranth genotypes under study. However, the correlation study revealed strong positive association of leaf area, shoot weight, shoot/root weight and stem base diameter with biological yield. Selection based on leaf area, shoot weight, shoot/root weight and stem base diameter

could lead to increase the biological yield of vegetable amaranth genotypes. Insignificant genotypic correlation was observed among mineral, quality and agronomic traits except K *vs.* Mg (0.753), protein *vs.* dietary fiber (–0.295) and stem base diameter *vs.* Ca (–0.491) indicated that selection for high mineral, protein and dietary fiber content might be possible without compromising yield loss. Based on mean performance of the genotypes, six vegetable amaranth genotypes VA16, VA27, VA26, VA14, VA18 and, VA13 were identified as high yielding having substantial mineral, protein and dietary fiber content.

Abstract

Forty-three vegetable amaranth (*Amaranthus tricolor* L.) genotypes selected from different eco-geographic regions of Bangladesh were evaluated during 3 years (2012-2014) for genetic variability, heritability and genetic association among mineral elements and quality and agronomic traits in randomized complete block design (RCBD) with five replications. The analysis showed that vegetable amaranth is a rich source of K, Ca, Mg, proteins and dietary fiber with average values among the 43 genotypes (1.014%, 2.476%, 2.984, 1.258% and 7.81%, respectively). Six genotypes (VA13, VA14, VA16, VA18, VA26, VA27) showed a biological yield >2000 g/m^2 and high mineral, protein and dietary fiber contents; eleven genotypes had high amount of minerals, protein and dietary fiber with above average biological yield; nine genotypes had below average biological yield but were rich in minerals, protein and dietary fiber. Biological yield exhibited a strong positive correlation with leaf area, shoot weight, shoot/root weight and stem base diameter. Insignificant genotypic correlation was observed among mineral, quality and agronomic traits, except K *vs.* Mg, protein *vs.* dietary fiber and stem base diameter *vs*. Ca. Some of these genotypes can be used for improvement of vegetable amaranth regarding mineral, protein and dietary fiber content without compromising yield loss.

2.2.2 Variability in total antioxidant capacity, antioxidant leaf pigments and foliage yield of vegetable amaranth

Purpose of the study

The interest of consumers in the aesthetic, nutritional and safety aspects of food has increased the demand for natural pigments such as chlorophyll, betalain, and carotene. Betalain are water-soluble compounds found in a limited number of families of the plant order Caryophyllales like *Amaranthus* have a unique source of betalain and important free radical-scavenging activity [30, 31]. betacyanin are red to purple colored betalain (condensation of betalamic acid and cyclo-Dopa, considering hydroxycinnamic acid derivatives or sugars as residue) and yellow colored betalain known as betaxanthin (imine condensation products between betalamic acid and amines or amino acid residues) [32]. Similarly, carotene grouped into alpha-carotene, beta-carotene and xanthophyll.

Pigments and their pharmacological activities include anticancer [40], antilipidemic [41] and antimicrobial [42] activities, indicating that betalain and carotene may be a potential source for the production of functional foods. Presently, the only commercial source of betalain and carotene is the red beet root. The colorant preparations from red beet root labelled as E-162 are exempted from batch certification. E-162 is used in processed foods such as dairy products and frozen desserts [34].

Among the naturally occurring vegetable pigments, betalain are rare and limited to a few edible vegetables such as red beet and amaranth, while chlorophylls are widely distributed in plant species [43]. The active ingredients of betalain and carotene provide anti-inflammatory property to our food and act as potential antioxidants and reduce the risk of cardiovascular disease and lung and skin cancers and is widely used as additive for food, drugs, and cosmetic products because of natural properties and absence of toxicity [44, 45].

In Americas, Japan, few Asian and European countries it is freshly intake by making salad or juice. It demands more genotypes enriched with leaf pigments. We found lots of variations in vegetable amaranth germplasm in respect to minerals, vitamins, leaf color, quality, and agronomic traits in our earlier studies [143, 149-151]. Therefore, to fill the lacuna, an investigation was carried out i) to estimate total antioxidant capacity, amount of antioxidant leaf pigments and foliage yield in 43 cultivated genotypes of vegetable amaranth, ii) to select appropriate high yielding genotypes containing high antioxidant leaf pigments for making colorful juice, commercially and (iii) to find out possible ways for improving the antioxidant leaf pigments without compromising foliage yield.

Material and methods

Seeds of 43 promising vegetable amaranth genotypes were selected in our previous studies of 102 genotypes, above selected genotypes were identified as promising due its high yield potential as well as variation in stem and leaf color. The genotypes were sown in a randomized complete block design (RCBD) with 3 replications, during three successive years ((2014 and 2015) under two sub experiments. Each accession was sown in 1 m^2 plot for both sub experiments. The spacing was 20 cm from row-to-row and 5 cm from plant-to-plant, respectively. Total compost (10 ton/ha) was applied during final land preparation. Urea, Triple super phosphate, muriate of potash and gypsum were applied at 200, 100, 150 and 30 kg/ha, respectively. Appropriate cultural practices were also maintained. Thinning was done to maintain appropriate plant density within rows. Weeding and hoeing was done at 7 days interval. Day temperature during experimental period ranged from 25 to 38°C. Irrigation was provided in 5-7 days interval. Data were collected at 30 days after seed sowing for foliage yield and antioxidant leaf pigments.

Data collection of foliage yield

Data were collected 30 days after sowing of seeds. The data were recorded on 10 randomly selected plants in each replication for foliage yield per plant in gram.

Determination of chlorophyll and total carotenoid content

Chlorophyll *a*, chlorophyll *b* and total chlorophyll were determined from 96% ethanolic extracts of the fresh-frozen amaranth leaves following Lichtenthaler and Wellburn [152] method and total carotenoid content was determined from acetone:haxen extract of the fresh-frozen amaranth leaves using spectrophotometer (Hitachi, U-1800, Tokyo, Japan) at 665, 649, and 470 nm for chlorophyll *a*, chlorophyll *b* and total carotenoid contents, respectively.

Determination of betacyanin and betaxanthin content

betacyanin and betaxanthin were extracted from fresh-frozen amaranth leaves using 80% methanol containing 50 mM ascorbic acid according to Wyler *et al.* [153]. betacyanin and betaxanthin were measured spectrophotometrically at 540 and 475 nm, respectively. The quantifications were done using mean molar extinction coefficients, which were 62×10^6 cm^2 mol^{-1} for betacyanin and 48×10^6 cm^2 mol^{-1} for betaxanthin. The results were expressed as nanograms betanin equivalent per gram fresh-frozen weight (FFW) for betacyanin and nanograms indicaxanthin equivalent per gram FFW for betaxanthin.

Determination of ascorbic acid

Ascorbic acid was measured following the procedure described in the previous chapter

Extraction of samples for chemical analysis

The leaves were harvested at the edible stage, 30 days after sowing, and dried overnight in an oven for chemical analysis. One gram of dried leaf from each cultivar was ground and dissolved in 40 mL of 90% methanol. The tightly capped bottle was then placed in shaking water bath (Thomastant T-N22S, Thomas Kagaku Co. Ltd., Japan) for 1 h. Then, the extract was filtered for further analytical assays of total antioxidant capacity.

Total antioxidant capacity (TAC)

Antioxidant activity was measured using the diphenyl-picryl-hydrazyl (DPPH) radical degradation method [47]. Briefly, 10 µL of leaf extract solution (in triplicate) was placed in test tubes along with 4 mL of distilled water and 1 mL of 250 micromole DPPH solution. The tubes were mixed and allowed to stand for 30 min in the dark before the absorbance was read at 517 nm using a spectrophotometer (U-1800, HITACHI, Tokyo, Japan). Antioxidant activity was calculated as the percent of inhibition relative to the control using the following equation:

Antioxidant activity (%) = (A blank - A sample/A blank) × 100

Where, A blank is the absorbance of the control reaction (10 µL of methanol instead of sample extract) and A sample is the absorbance of the test compound. Trolox was used as the reference standard, and the results were expressed as µg trolox equivalent g^{-1} dw.

Statistical analysis

The raw data of consecutive two years were compiled by taking the means of all the plants taken for each treatment and replication for different traits. The mean data of consecutive two years were averaged and the averages of two years means were statistically and biometrically analyzed. Analysis of variance was done according to Panse and Sukhatme [132] for each character. Genotypic and phenotypic variances, genotypic (GCV) and phenotypic coefficient of variation (PCV), heritability (h^2_b) in broad sense, and genetic advance in percent of mean (GAMP, %) and correlation were estimated according to Singh and Chaudhary [133].

Results and discussion

Mean performance

Mean performance, coefficient of variation (CV%) and critical difference (CD) of leaf pigments and foliage yield for 43 vegetable amaranth genotypes are presented in Table 1. The analysis of variance revealed significant differences among the genotypes for all the 10 traits, indicating the validity of further statistical analysis (Table 1).

Leaf pigments serves as an antioxidant help to protect many diseases including cancer, cardiovascular diseases, neurodegenerative diseases and inflammation and prevent aging [6].

Chlorophyll a

In statistical analysis, the chlorophyll *a* content had significant pronounced variations among the genotypes. Accession VA13 had the highest chlorophyll *a* content (636.87 µg g^{-1}), followed by VA19 (523.21 µg g^{-1}), VA14 (517.16 µg g^{-1}), and VA16 (504.56 µg g^{-1}). The lowest amount of chlorophyll *a* was found in VA34 (126.47 µg g^{-1}). Eighteen genotypes showed above average mean values for chlorophyll *a* content. The mean chlorophyll *a* content was 290.18 µg g^{-1}. The estimated CV for chlorophyll *a* was 2.41%.

Chlorophyll b

Accession VA17 had the highest chlorophyll *b* content (292.19 µg g^{-1}), followed by VA7 (278.21 µg g^{-1}), VA15 (271.08 µg g^{-1}) and VA13 (268.34 µg g^{-1}). The lowest amount of chlorophyll *b* was found in VA29 (49.63 µg g^{-1}). The mean chlorophyll *b* content was 142.54 µg g^{-1}. Seventeen genotypes showed above average mean values for chlorophyll *b* content. The estimated CV for chlorophyll *b* was 2.61%.

Total Chlorophyll

The total chlorophyll content showed a highly pronounced variation among all the chlorophyll traits. VA13 had the highest total chlorophyll content (906.23 µg g^{-1}), followed by VA14 (770.22 µg g^{-1}), VA7 (753.73 µg g^{-1}), VA16 (737.43 µg g^{-1}), VA15 (704.83 µg g^{-1}) and VA19 (703.04 µg g^{-1}). The lowest amount of total chlorophyll was found in VA34 (193.31 µg g^{-1}). Seventeen genotypes showed above average mean values for total chlorophyll content. The estimated CV for total chlorophyll was 1.83%.

Betacyanin

There were significant variations among the genotypes in betacyanin contents and the average betacyanin content was 302.68 ng g^{-1}. The highest betacyanin content was observed in VA18 (538.51 ng g^{-1}), followed by VA3 (537.21 ng g^{-1}), and VA14 (500.40 ng g^{-1}), while the lowest betacyanin content was observed in VA29 (106.37 ng g^{-1}). The CV of this trait was 1.85%. Out of 43 genotypes, 15 genotypes showed above-average values for betacyanin content.

Betaxanthin

There were significant variations among the genotypes in betaxanthin contents. The average betaxanthin content was 306.93 ng g^{-1}. The highest betaxanthin content was observed in VA3 (584.71 ng g^{-1}), followed by VA18 (554.31 ng g^{-1}), VA14 (502.79 ng g^{-1}), and VA16 (492.99 ng g^{-1}), while the lowest betaxanthin content was observed in VA29 (99.94 ng g^{-1}). The CV was 2.29%. 19 genotypes showed above-average values for betaxanthin content.

Table 1. Mean performance, %CV and CD for total antioxidant capacity, antioxidant leaf pigments and vitamins, foliage yield in vegetable amaranth genotypes

Genotypes	Chlorophyll *a* (µg g^{-1})	Chlorophyll *b* (µg g^{-1})	Total chlorophyll (µg g^{-1})	Beta cyanin (ng g^{-1})	Beta xanthin (ng g^{-1})	Betalain (ng g^{-1})	Total carotene (mg 100 g^{-1})	Ascorbic acid (mg 100 g^{-1})	TAC (TEAC µg g^{-1} dw)	Foliage yield $plant^{-1}$ (g)
VA1	174.54	83.06	258.61	286.85	256.38	543.16	76.02	175.59	18.62	8.94
VA2	346.84	158.54	506.39	391.53	398.09	789.55	83.89	11.97	30.95	14.62
VA3	304.82	226.20	532.03	537.21	584.71	1121.85	55.38	16.34	32.83	15.36
VA4	160.74	66.74	228.49	249.15	268.76	517.84	69.04	96.49	18.92	9.20
VA5	358.73	156.37	516.12	356.29	358.17	714.38	72.07	63.69	27.65	12.12
VA6	131.56	62.42	194.99	185.52	181.90	367.35	68.84	87.17	15.64	7.32
VA7	474.51	278.21	753.73	279.76	281.07	560.75	55.47	102.81	17.68	9.14
VA8	127.26	51.67	179.94	340.28	344.11	684.32	56.18	101.65	14.99	12.58
VA9	381.33	190.65	573.00	427.66	417.25	844.84	73.52	71.85	29.85	15.60
VA10	240.90	106.45	348.36	264.03	274.70	538.66	44.81	134.61	18.62	12.30
VA11	205.99	222.16	429.16	385.52	372.19	757.64	88.29	135.58	29.98	12.12
VA12	131.07	71.27	203.35	233.87	230.57	464.36	65.91	97.70	32.02	9.80
VA13	636.87	268.34	906.23	407.94	427.55	835.42	32.77	94.49	32.82	16.14
VA14	517.16	252.05	770.22	500.40	502.79	1003.12	65.55	184.77	27.68	32.06
VA15	432.74	271.08	704.83	343.99	346.18	690.10	59.81	66.63	24.98	23.28
VA16	504.56	231.86	737.43	484.77	492.99	977.69	82.89	72.01	28.61	26.46
VA17	400.18	292.19	693.38	225.64	218.70	444.27	96.08	113.97	18.63	13.20
VA18	429.62	239.09	669.72	538.51	554.31	1092.74	49.39	67.69	29.93	26.40
VA19	523.21	178.82	703.04	302.17	308.31	610.41	96.37	96.49	31.68	19.20
VA20	441.60	217.78	660.39	453.59	467.36	920.87	105.08	65.69	32.65	23.14
VA21	131.46	64.19	196.66	152.26	171.77	323.95	123.91	91.79	16.28	13.48
VA22	204.40	57.62	263.03	284.63	294.60	579.16	132.32	87.59	12.80	18.54
VA24	254.38	93.33	348.72	228.75	252.75	481.42	125.17	36.89	10.18	15.48
VA25	360.71	120.97	482.69	352.26	364.29	716.47	113.38	58.53	11.54	10.88
VA26	176.83	77.09	254.94	301.49	311.15	612.57	118.80	84.17	9.21	18.64
VA27	238.91	124.47	364.40	203.95	226.51	430.39	91.27	82.27	15.17	26.50
VA28	172.75	97.55	271.31	134.51	129.40	263.84	116.76	18.87	16.14	22.04
VA29	170.52	49.63	221.16	106.37	99.94	206.23	117.41	185.89	20.14	15.14
VA30	295.19	170.28	466.49	330.52	337.47	667.92	97.15	58.53	16.80	9.61
VA31	257.87	83.50	342.38	252.70	249.25	501.88	112.35	84.33	12.78	6.45
VA32	230.69	78.52	310.23	177.54	182.72	360.18	113.68	46.67	13.35	7.88
VA33	200.52	91.90	293.44	256.25	250.48	506.65	125.32	42.36	14.55	12.54
VA34	126.47	65.83	193.31	238.51	246.30	484.73	93.61	65.69	11.25	10.87
VA35	221.61	100.62	323.24	246.05	243.32	489.29	114.95	45.25	10.58	13.64
VA36	242.90	105.79	349.70	211.69	236.92	448.54	102.89	132.45	12.78	12.46
VA37	221.27	211.93	434.22	241.17	245.42	486.51	129.30	67.85	14.55	11.12
VA38	348.06	189.41	538.49	338.95	326.77	665.65	112.79	49.06	8.90	16.96
VA39	154.75	68.71	224.47	315.88	319.20	635.01	68.19	64.69	8.92	14.76
VA40	208.30	58.07	267.38	177.09	176.16	353.18	104.52	36.05	12.47	9.20
VA41	231.02	125.33	357.36	161.57	167.96	329.46	96.37	106.81	13.35	12.88
VA42	303.89	172.64	477.54	298.38	254.10	552.41	62.21	54.37	16.28	18.73
VA43	380.80	130.01	511.83	289.51	282.39	571.83	117.67	86.17	14.85	13.94
VA44	251.86	50.72	303.37	349.15	370.76	719.84	123.04	102.65	20.11	11.86
Mean	290.175	142.54	433.72	302.675	306.925	609.53	89.565	83.145	21.71	15.01
F values	**	**	**		**	**	**	**	**	**
SE	0.135	0.1478	0.1709	0.50585	0.5229	0.4939	0.14895	0.22985	0.00305	0.03276
CV%	2.411	2.6125	1.832	1.848	2.2875	1.9275	1.5955	2.1025	0.11275	2.154
CD	0.2832	0.3084	0.40665	1.2765	1.3038	1.2395	0.3157	0.48315	0.00395	0.0778

TAC, total antioxidant capacity; dw, dry weight; TEAC, trolox equivalent antioxidant capacity. *, **, significant at 5% level and 1% level, respectively.

Betalain

There were significant variations among the genotypes in betalain contents. The average betalain content was 609.53 ng g^{-1}. The highest betalain content was observed in VA3(1121.85

ng g^{-1}) followed by VA18 (1092.74 ng g^{-1}), VA14 (1003.12 ng g^{-1}), VA16 (977.69 ng g^{-1}), and VA20 (920.87 ng g^{-1}), while the lowest betalain content was observed in VA29 (206.23 ng g^{-1}). The CV was 1.92%. Out of 43 genotypes, 16 showed above-average values for betalain content.

Total carotene

There were significant variations among the genotypes in total carotene contents. The average total carotene content was 89.57 mg 100 g^{-1}. The highest total carotene content was observed in VA22 (132.32 mg 100 g^{-1}), followed by VA24, VA33, VA37, VA21, and VA44, while the lowest total carotene content was observed in VA42 (62.21 mg 100 g^{-1}). The CV of this trait was 1.60%. Out of 43 genotypes, 23 showed above-average values for total carotene content.

Ascorbic acid

There were significant variations among the genotypes in ascorbic acid contents. The average ascorbic acid content was 83.15 mg 100 g^{-1}. The highest ascorbic acid content was observed in VA29 (185.87 mg 100 g^{-1}), followed by VA14, VA1, VA11, VA36, VA41, and VA44, while the lowest ascorbic acid content was observed in VA2 (11.97 mg 100 g^{-1}). The CV was 2.01%. Out of 43 genotypes, 17 genotypes showed above-average values for ascorbic acid content.

Total antioxidant capacity (TAC)

The variations of TAC were highly pronounced among the genotypes which ranged from 8.90 TEAC µg g^{-1} dw (VA38) to 32.83 TEAC µg g^{-1} dw (VA3). The highest TAC was found in the genotype VA3 (32.83 TEAC µg g^{-1} dw) VA13 (32.82 TEAC µg g^{-1} dw) and VA20 (32.65 TEAC µg g^{-1} dw) followed by VA12 (32.02 TEAC µg g^{-1} dw), VA19 (31.68 TEAC µg g^{-1} dw), VA2 (30.95 TEAC µg g^{-1} dw), VA11 (29.98 TEAC µg g^{-1} dw), VA18 (29.93 TEAC µg g^{-1} dw) and VA9 (29.85 TEAC µg g^{-1} dw). In contrast, the lowest TAC was observed in VA38 (8.90 TEAC µg g^{-1} dw). The average mean of TAC was 21.27 TEAC µg g^{-1} dw. Thirteen genotypes showed above-average performance for TAC. The coefficient of variation for this trait was 0.112%.

Foliage yield

It had significant and the highest variations among the genotypes. The highest value was found in VA14 (32.06 g) followed by VA18 (26.40 g), VA16 (26.46 g), VA15 (23.28 g), and VA20 (23.14 g). The lowest value was observed in VA6 (7.32 g) followed by VA1 (8.94 g), VA4 (9.14 g) and VA7 (9.20 g). The average was 15.95 g. The CV (2.15) was low in this trait analyzed. Seven genotypes showed above-average values.

The present investigation revealed that vegetable amaranth is rich in chlorophyll *a* (290.16 µg g^{-1}), chlorophyll *b* (142.54 µg g^{-1}), Total chlorophyll (433.72 µg g^{-1}), betacyanin

(302.68 ng g^{-1}) and betaxanthin (306.93 ng g^{-1}), betalain (609.53 ng g^{-1}), total carotene (89.57 mg 100 g^{-1}) ascorbic acid (83.15 mg 100 g^{-1}) and total antioxidant (21.71 TEAC µg g^{-1} dw).

Five genotypes, VA14, VA16, VA18, VA15, and VA20 showed high foliage yield and also found to be a rich source of antioxidant leaf pigments and vitamins. Selection of these genotypes would be economically useful for antioxidant leaf pigments and vitamins, and high yield aspects. The genotypes VA13 and VA19 had above average foliage yield along with rich source of the antioxidant leaf pigments and vitamins while the genotypes VA2, VA3, VA9, VA11, VA12 and VA17 had a high amount of the colorant antioxidant leaf pigments and below-average foliage yield. These eight genotypes can be used as a donor parent for integration of potential genes of the high antioxidant leaf pigments and vitamins into other genotypes.

Variability studies

The genotypic and phenotypic variance (σ^2g, σ^2p), coefficients of variation (GCV, PCV), h^2_b, GA and GAMP are presented in Table 2. The highest genotypic variance was observed for betalain (20318.65), followed by total chlorophyll (10522.15), betaxanthin (5157.75), betacyanin (5116.08), chlorophyll *a* (4684.08), chlorophyll *b* (2106.41) indicating greater scope of selection for these traits. Ascorbic acid (1311.99), total carotene (321.32), TAC (42.09) and foliage yield (2,52) exhibited moderate genotypic variances.

Table 2. Genetic parameter for total antioxidant capacity, antioxidant leaf pigments and vitamins, foliage yield in vegetable amaranth genotypes

Genetic parameter	Chlorophyll *a* (µg g^{-1})	Chlorophyll *b* (µg g^{-1})	Total chlorophyll (µg g^{-1})	Beta cyanin (ng g^{-1})	Beta xanthin (ng g^{-1})	Betalain (ng g^{-1})	Total carotene (mg 100 g^{-1})	Ascorbic acid (mg 100 g^{-1})	TAC (TEAC µg g^{-1} dw)	Foliage yield $plant^{-1}$ (g)
σ^2g	4684.08	2106.41	10522.15	5116.08	5157.75	20318.65	321.32	1311.99	42.09	21.52
σ^2p	4750.25	2215.25	10835.62	5242.63	5345.65	20762.35	355.26	1402.72	44.17	24.48
GCV	19.77	25.32	19.41	19.88	19.71	19.69	25.66	39.01	25.20	29.08
PCV	19.91	25.97	19.70	20.13	20.07	19.90	26.98	40.33	25.82	31.02
h^2_b	99.30	97.51	98.54	98.79	98.23	98.93	95.10	96.71	97.62	93.76
GA	140.99	94.55	211.31	147.35	147.94	293.64	36.93	74.62	13.36	9.56
GAMP	40.72	52.16	39.99	40.96	40.61	40.56	52.85	80.35	51.92	59.91

TAC, Total antioxidant capacity; dw, Dry weight; TEAC, Trolox equivalent antioxidant capacity; σ^2g, Genotypic variance; σ^2p, Phenotypic variance; GCV, Genotypic coefficient of variation; PCV, phenotypic coefficient of variation; h^2_b, Heritability in broad sense; GAMP, Genetic advance in percent of mean

The phenotypic variances for all the traits were slightly higher but close to the genotypic variances which indicated the predominance of additive gene actions. GCV values ranged from 19.41 (total chlorophyll) to 29.08% (foliage yield). The PCV values ranged from 19.70% (total chlorophyll) to 40.33% (ascorbic acid). In the present investigation, all the traits had high to moderate genotypic and phenotypic variances along with moderate GCV and PCV values,

which indicate scope for improvement in these traits through selection due to predominance of additive gene action for these traits. The heritability estimates were high for all the traits and ranged from 93.76% (foliage yield) to 99.30% (chlorophyll *a*). The highest expected genetic advance was exhibited for betalain (293.64), followed by total chlorophyll (211.31%) betaxanthin (147.94), betacyanin (147.35%), chlorophyll *a* (140.99%), and chlorophyll *b* (94.44%). Genetic advance in percent of the mean (GAMP) ranged from 40.72 (chlorophyll *a*) to 80.35 (ascorbic acid). The highest GAMP was found in ascorbic acid (80.35%), followed by foliage yield (59.91%), total carotene (52.85) Chlorophyll *b* (52.16%), TAC (51.92), chlorophyll *a*, total chlorophyll, betacyanin, betaxanthin, and betalain showed moderate GAMP (around 40%). In the present study, the high heritability and high to moderate genetic advance values were observed for all the traits indicated preponderance of additive gene effects and improvement could be achieved through selection of these traits.

Correlation studies

The phenotypic and genotypic correlations between the various characters are presented in Table 3. In the present investigation, the genotypic correlation coefficients were very much close to the corresponding phenotypic values for all the traits that indicating predominance of additive gene action i. e., less environmental influence of these traits. The chlorophyll *a* had a significant positive correlation with all the traits except total carotene and ascorbic acid.

Chlorophyll *b* exhibited significant positive correlation with total chlorophyll and TAC. Similarly, total chlorophyll had a significant positive interrelationship with all the traits except total carotene and ascorbic acid. A similar trend of positive associations was observed by earlier work in *A. tricolor* [7, 143]. betacyanin had a significant positive association with betaxanthin, betalain and TAC. betaxanthin showed significant positive associations with betalain and TAC. Similarly, betalain exerted positive interrelationships with TAC. Total antioxidant capacity showed significant positive associations with all the leaf pigments, ascorbic acid and foliage yield. These indicates that high antioxidant content was closely associated with foliage yield of vegetable amaranth. On the other hand, foliage yield had insignificant correlation with all the leaf pigments, ascorbic acid. These indicate that improvement of foliage yield, ascorbic acid, antioxidant leaf pigments might be possible by improving any of the antioxidant leaf pigments. Shukla *et al.* [23] observed a positive association of foliage yield with beta carotene and ascorbic acid. Interesting results is that, ascorbic acid and total carotene had an insignificant negative and negligible interrelationship among all antioxidant vitamin and leaf pigments while it exhibited significant positive associations with total antioxidant capacity.

Table 3. Genotypic and phenotypic correlation co-efficient (r_g and r_p) for total antioxidant capacity, antioxidant leaf pigments and vitamins, foliage yield in vegetable amaranth genotypes

Traits		Chlorophyll *b* (µg g^{-1})	Total chlorophyll (µg g^{-1})	Beta cyanin (ng g^{-1})	Beta xanthin (ng g^{-1})	Betalain (ng g^{-1})	Total carotene (mg 100 g^{-1})	Ascorbic acid (mg 100 g^{-1})	TAC (TEAC µg g^{-1} dw)	Foliage yield (g)
Chlorophyll *a* (µg g^{-1})	r	0.594**	0.933**	0.545**	0.491*	0.521**	-0.032	-0.132	0.482*	-0.077
	r	0.592**	0.932**	0.542**	0.490*	0.520**	-0.030	-0.131	0.480*	-0.076
Chlorophyll *b* (µg g^{-1})	r		0.844**	0.315	0.240	0.279	-0.186	-0.253	0.402*	0.099
	r		0.842**	0.314	0.238	0.278	-0.185	-0.252	0.400*	0.098
Total chlorophyll (µg g^{-1})	r			0.504*	0.435*	0.472*	-0.105	-0.202	0.472*	-0.008
	r			0.503*	0.433*	0.471*	-0.104	-0.201	0.471*	-0.007
Betacyanin (ng g^{-1})	r				0.978**	0.994**	-0.132	-0.240	0.651**	-0.083
	r				0.976**	0.992**	-0.131	-0.238	0.651**	-0.082
betaxanthin (ng g^{-1})	r					0.995**	-0.052	-0.194	0.652**	-0.095
	r					0.994**	-0.051	-0.193	0.651**	-0.094
Betalain (ng g^{-1})	r						-0.093	-0.218	0.654**	-0.089
	r						-0.092	-0.217	0.653**	-0.088
Total carotene (mg 100 g^{-1})	r							0.063	0.557**	-0.142
	r							0.062	0.556**	-0.140
Ascorbic acid (mg 100 g^{-1})	r								0.792**	-0.011
	r								0.786**	-0.010
TAC (TEAC µg g^{-1} dw)	r									0.485*
	r									0.484*

* Significant at 5% level, ** Significant at 1% level

Considering high genotypic and phenotypic variances along with GCV and PCV values, high heritability coupled with GAMP, all the traits except foliage yield could be selected for the improvement of 43 vegetable amaranth genotypes under study. However, the correlation study revealed a strong positive association among all the antioxidant leaf pigments and total antioxidant capacity. Selection based on antioxidant leaf pigments and total antioxidant capacity could economically viable to improve the antioxidant potential of vegetable amaranth genotypes. Insignificant negative genotypic correlation was observed between total carotene versus all antioxidant leaf pigments, ascorbic acid versus all antioxidant leaf pigments and foliage yield versus rest of all traits. This indicates that selection for antioxidant leaf pigments and ascorbic acid content might be possible without compromising yield loss. The genotype VA14, VA16, VA18, VA15, and VA20 could be selected as an antioxidant leaf pigments and vitamins enriched high-yielding vegetable amaranth varieties to produce juice. The genotypes VA13 and VA19 had above average foliage yield and enrich of antioxidant profiles while the genotypes VA2, VA3, VA9, VA11, VA12 and VA17 had a high antioxidant profiles and below-average foliage yield. These eight genotypes can be used as a donor parent for integration of potential genes of the high antioxidant leaf pigments into other genotypes.

Abstract

Forty-three vegetable amaranth genotypes were evaluated for total antioxidant capacity, antioxidant leaf pigments, vitamins and selection of suitable genotypes for extraction of juice in a randomized complete block design (RCBD) with three replications. Vegetable amaranth was rich in chlorophyll, betacyanin, betaxanthin, betalain, carotene, ascorbic acid and total antioxidant. The genotype VA14, VA16, VA18, VA15, and VA20 could be selected as an antioxidant leaf pigments and vitamins enriched high-yielding vegetable amaranth varieties to produce juice. The genotypes VA13 and VA19 had above average foliage yield and high antioxidant profiles while the genotypes VA2, VA3, VA9, VA11, VA12, and VA17 had a high antioxidant profiles and below-average foliage yield. These genotypes could be used as a donor parent for integration of potential high antioxidant profiles genes into other genotypes. The correlation study revealed a strong positive association among all the antioxidant leaf pigments *vs* total antioxidant capacity and foliage yield *vs* total antioxidant capacity. Selection based on total antioxidant capacity, antioxidant leaf pigments could economically viable to improve the yield potential of vegetable amaranth genotypes. Total carotene and ascorbic acid exhibited insignificant genotypic correlation with all the traits except total antioxidant capacity. This indicates that selection for antioxidant vitamins might be possible without compromising yield loss.

2.2.3 Phenotypic divergence in vegetable amaranth for total antioxidant capacity, antioxidant profile, dietary fiber, nutritional and agronomic traits

Purpose of the study

Antioxidant vitamins and minerals include vitamins A, C, and E; beta-carotene; and the minerals selenium, zinc, manganese, copper, and iron [52, 53. Sufficient delivery of the first line defense antioxidants (Cu, Zn, Fe and Mn) from diet is required in order for the body to synthesize antioxidant metalloenzymes such as catalase (Fe) and superoxide dismutase (Cu, Zn, and Mn) [52]. Antioxidant vitamins and minerals, phenolic compounds and flavonoids protect the body from harmful free radicals that can cause damage to cells and impair the immune system and lead to infections and various degenerative diseases like heart disease, neuro-degenerative disease, atherosclerosis, cancer, arthritis, cataracts, emphysema, retinopathy. [49, 52].

Amaranth is exceedingly adaptable to adverse growing conditions and has no major disease problems. It is essential to know the status of antioxidant content, polyphenol, flavonoid, antioxidant vitamins and minerals, dietary fiber, nutritional and agronomic traits and address genetic augmentation for improving the foliage and biological yield of vegetable amaranth along with its antioxidant profile, nutrient, protein, and dietary fiber contents.

Genetic diversity assessment is a useful tool to help breeders for identifying appropriate parental combinations for the creation of suitable segregating progenies with excellent genetic variability that also facilitates integration of desirable genes from a diverse germplasm into the existing genetic base population [29]. Multivariate statistical methods have been successfully used to classify both quantitative and qualitative variation in many crop species, including mustard [56], Russian wild rye [57], *Arachis* [58] and Ethiopian mustard [59]. There are few reports on genetic diversity in grain amaranth [60-62]; however, Shukla *et al.* [63] performed a diversity analysis on *Amaranthus tricolor* for nutrient content and agronomic traits. Therefore, we assessed the status and magnitude of diversity of vegetable amaranth for its antioxidant profile in combination with nutrient, dietary fiber and agronomic traits and augmented these traits towards foliage and biological yield. This study was conducted with the following purposes i) to know the status of antioxidant profile, dietary fiber and nutrient contents, and yield and the contribution of agronomic traits and attain a meaningful grouping of vegetable amaranth genotypes based on the contribution of those traits to divergence. And ii) to determine appropriate genotypic groups for efficient and proper utilization of germplasm in future breeding programs.

Materials and methods

Experimental site

The experiment was conducted at the experimental field of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh.

Materials, design, layout, and cultural practices

Forty-three distinct and promising genotypes of vegetable amaranth (Accession number 1-43) were investigated for two successive years (2014 and 2015). Experiment was carried out in a randomized complete block design (RCBD) with three replications. The unit plot size of each genotype was 2 m^2. The spacing was 20 cm between rows and 5 cm between plants. Recommended fertilizer and compost doses and appropriate cultural practices were maintained.

Data collection of agronomic traits

Data were collected 30 days after sowing the seeds for both years. The data were recorded on 10 randomly selected plants in each replication for agronomic traits, including plant height (cm), leaves per plant, leaf area (cm^2), shoot weight (g), shoot: root ratio, and stem base diameter (cm). Foliage and biological yield were harvested on a whole-plot basis.

Estimation of beta-carotene, vitamin C, protein, dietary fiber

Estimation of beta-carotene, vitamin C, protein and dietary fiber were measured following the procedure described in the previous chapter

Extraction of samples for total polyphenol content, total flavonoids content, total antioxidant activity.

Samples were extracted following the procedure described in the previous chapter

Determination of total polyphenols content (TPC)

The total phenolic content of red amaranth was determined using the Folin-Ciocalteu reagent method described by Slinkard and Singleton [154] with gallic acid as a standard phenolic compound. Briefly, 50 µl of the leaf extract solution was placed in a test tube along with1 ml of Folin-Ciocalteu reagent (previously diluted 1:4, reagent: distilled water) and then mixed thoroughly. After 3 min, 1 ml of $NaCO_3$ (10%) was added, and the mixture allowed to stand for 1 h in the dark. The absorbance was measured at 760 nm using a spectrophotometer (U-1800, HITACHI, Tokyo, Japan). The concentration of total phenolic compounds in the leaf extracts was determined using an equation obtained from a standard gallic acid graph. The results are expressed as mg gallic acid equivalent (GAE) kg^{-1} dw.

Determination of total flavonoid content (TFC)

The total flavonoid content in vegetable extract was determined using the aluminum chloride colorimetric method described by Chang *et al*. [155]. For this assay, 500 µl of leaf extract was transferred to a test tube along with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After 30 min at room temperature, the absorbance of the reaction mixture was measured at 415 nm using a spectrophotometer (U-1800, HITACHI, Tokyo, Japan). Rutin was used as the standard compound, and TFC is expressed as mg rutin equivalent (RE) kg^{-1} dw.

Total antioxidant capacity (TAC)

Total antioxidant capacity was measured following the procedure described in the previous chapter

Statistical analyses

The raw data were compiled by taking the means of all plants from each treatment in both experimental years. The pooled means of both years were then subjected to further statistical and biometrical analyses, including analysis of variance (ANOVA), as described by Singh and Chaudhary [133]. The mean data were standardized and subjected to a multivariate analysis of numerical taxonomic techniques using the procedure of principal component analysis (PCA) [156]. To group the 43 vegetable amaranth genotypes and to elucidate patterns of similarity and dissimilarity, the data were subjected to cluster analysis using Ward's method (Ward, [157].

Results and discussion

Antioxidant vitamins and minerals, TPC and TFC of vegetable amaranth remove free radicals from the body and help to fight against infections and other conditions including cancer, coronary artery diseases, muscular degeneration and serious eye diseases [8]. The contents of beta-carotene, vitamin C, Fe, Zn, Cu and Mn are the most important antioxidant traits of vegetable amaranth [11, 13, 15, 52, 54]. On the other hand, amaranth can relieve vitamin and nutrient deficiency in the human diet. Anemia, night blindness, scurvy, rickets and protein deficiency are serious problems for children in poor communities in third-world countries, including the Indian subcontinent. Therefore, vegetable amaranth might be an excellent source of antioxidants, nutrients and dietary fiber.

Vegetable amaranth genotypes exhibited highly significant differences with a high degree of variation in total antioxidant capacity, antioxidant profile, dietary fiber, and nutritional and agronomic traits in both years.

Principal component analysis

Principal component analysis (PCA) was performed by simultaneously considering total antioxidant capacity and all antioxidant profile, dietary fiber, and nutritional and agronomic traits to evaluate deviation patterns among 22 variables consecutively. The characters were initially scaled to make their variances equal. In the multivariate space in which they are defined, a new set of axes was chosen such that the variance on each axis was as large as possible but at right angles to the preceding ones. The coefficient of each data point on each new axis was a weighted sum of its coefficients on the original axes. The variance on each axis is called the latent root and the percentage of the total variance that each represents and the coefficients used in the weighted sum (loadings or eigen vectors) for 22 antioxidants, nutrients or agronomic traits in the 43 genotypes are presented in Table 1.

Table 1. Eigen values, proportion of variability, antioxidant profile, dietary fiber, nutrient and agronomic traits contributing to the first four PCs of 43 vegetable amaranth genotypes.

		PC_1	PC_2	PC_3	PC_4
	Root	225530.90	134546.10	54.387.13	3828.26
	% variance explained	53.17	31.72	12.82	0.90
	Cumulative variance	53.17	84.89	97.71	98.61
		Coefficients of variates			
Antioxidant profile	Fe (µg g^{-1})	120.67	354.05	2.66	3.32
	Mn (µg g^{-1})	1.17	9.21	44.26	-10.69
	Cu (µg g^{-1})	1.28	-1.54	0.94	-1.63
	Zn (µg g^{-1})	56.87	-17.72	227.01	2.31
	Beta carotene (mg kg^{-1})	-11.81	3.68	0.75	-5.71
	Vitamin C (mg kg^{-1})	0.12	-3.46	-3.52	14.65
	TAC (TEAC mg kg^{-1}dw)	1.99	1.50	-1.38	0.80
	TPC (GAE mg kg^{-1}dw)	-0.20	-0.64	-1.54	1.37
	TFC (RE mg kg^{-1}dw)	7.03	1.89	-2.56	10.51
Nutrient traits	K (g 100 g^{-1})	0.02	0.02	0.04	-0.00
	Ca (g 100 g^{-1})	-0.08	0.01	0.08	-0.09
	Mg (g 100 g^{-1})	0.01	0.03	0.03	0.01
	Protein (g 100 g^{-1})	0.04	0.01	-0.03	0.03
	Dietary fiber (g 100 g^{-1})	-0.15	0.02	-0.03	0.04
Agronomic traits	Plant height (cm)	4.19	-2.63	-0.62	5.52
	Leaves $plant^{-1}$	1.25	-0.04	-0.91	1.50
	Leaf area (cm^2)	17.91	-22.74	-1.62	57.14
	Shoot weight (g)	4.82	-0.96	-0.34	-0.04
	Shoot: root ratio	5.16	0.98	1.76	-3.44
	Stem base diameter (cm)	0.56	-0.61	-0.38	0.90
	Foliage yield (kg)	144.44	-31.04	-6.44	2.56
	Biological yield m^{-2} (kg)	431.60	-85.26	-28.45	-4.76

The first two principal components (PCs) contributed 84.89%, and the first three PCs contributed 97.71% of the variability seen among the 43 vegetable amaranth genotypes for the traits under investigation. PC_1 accounted for 53.17% of the variation. In the present study, we found that four PCs account for 98.61% of the total variation present among the 43 genotypes of amaranth, indicating that the selected antioxidant, nutrient, and agronomic traits significantly contributed to the diversity of vegetable amaranth. Shukla *et al.* [63] observed

that 68% of the total variation for 16 morphological and nutritional traits was found in the first four PCs among 39 vegetable amaranth strains. PC_1 exhibited the highest positive coefficient of variation for biological yield. PC_1 also had the largest positive coefficients for foliage yield, iron, zinc, leaf area, total flavonoid content (TFC), shoot: root ratio, shoot weight, plant height, total antioxidant capacity (TAC), copper, leaves plant-1 and manganese content whereas, this PC showed negative coefficients for beta carotene, total polyphenol content (TPC) and dietary fiber. PC_1 had a positive coefficient for all of the traits except beta-carotene, TPC, calcium and dietary fiber. PC_2, accounted for 31.72% of the variation, had the highest positive coefficient for iron and high positive coefficients for manganese, beta-carotene, TFC, and TAC. PC_2 also had the largest negative coefficient for biological yield, followed by foliage yield, leaf area, and zinc. In contrast, PC_2 had high negative coefficients for vitamin C, plant height, copper, and shoot weight. PC_3 contributed 12.82% of the genetic variation and had the highest positive coefficient of variation for zinc. PC3 had the largest positive coefficient for manganese, iron, shoot: root ratio, copper and beta-carotene. In contrast, PC_3 had the highest negative coefficients for biological yield, foliage yield, vitamin C, TFC, leaf area, TPC, TAC, leaves $plant^{-1}$ and plant height. Finally, PC_4 contributed only 0.90% of the total genetic variation. PC_4 had the largest positive coefficient for leaf area and high positive coefficients for vitamin C, TFC, plant height, iron, foliage yield, zinc, TPC and leaves $plant^{-1}$. PC_4 also had high negative coefficients for manganese, beta-carotene, biological yield, shoot: root ratio, and copper content. All of the nutrient traits and dietary fiber for PC_4 had non-significant coefficients of variation, indicating less contribution of these traits towards genetic divergence of the 43 vegetable amaranths. The results from four PCs revealed that the foliage and biological yield had a close association with all agronomic traits, indicating that a tall, thick plant having much broader leaves, heavy shoots and a high shoot: root ratio significantly increases the foliage and biological yield of the vegetable amaranth. A previous report on Amaranthus by Shukla *et al*. [63] found similar results in PC_2 and PC_3 but differed from the results we observed in PC_1 and PC4. They found that PC1 grouped the genotypes with high foliage yield but with smaller leaves $plant^{-1}$ and PC4 grouped the genotypes with low foliage yield but broad and higher leaves $plant^{-1}$ which may be due to the high environmental influence of related traits on foliage yield or sampling error during data collection. Although Shukla *et al.* [63] extensively investigated nutritional and morphological traits in vegetable amaranth but this is the first report of diversity study on antioxidant profile such as TPC, TFC, and TAC in combination with antioxidant vitamins, minerals, dietary fiber and agronomic traits in vegetable amaranth. Thus, the results of the antioxidant profile show that TFC has the highest contribution to TAC

compared to mineral and vitamin antioxidants. Moreover, PC_1 and PC_4 distinguished those genotypes with high foliage yield, and the related agronomic traits were closely associated with high antioxidant profiles. PC_2 and PC_3, however, distinguished genotypes that had low foliage and biological yield and related traits and were also associated with a high antioxidant profile; hence, all genotypes had a high antioxidant profile. Therefore, high-yielding genotypes (especially from cluster VI) could be directly used as high antioxidant profile varieties, and low-yielding genotypes could be used as a source of donor parents in hybridization programs. All of the nutrient traits and dietary fiber results were of interest because none of the traits had a significant coefficient of variation in either the positive or negative direction, indicating less contribution of these traits to genetic divergence, but the highest contribution came from antioxidant profiles and agronomic traits.

Cluster analysis

The dendrogram of 43 vegetable amaranth genotypes for 22 antioxidant, nutrient and agronomic traits showed that the germplasm could be broadly divided into six clusters each carrying the amaranth genotype and sharing a common gene pool. following Ward's method [157] (Fig. 1). Shukla *et al*. [63] observed six clusters in 39 vegetable amaranth genotypes, while Pandey and Singh [62] found 18 clusters in 98 grain amaranth genotypes. However, Pandey [61] divided 26 grain amaranth genotypes into 11clusters. The mean values of the genotypes in each cluster are presented in Table 2. Cluster I included 13 genotypes enriched with manganese, copper, calcium, and magnesium and had a higher biological yield. Genotypes from cluster I had a moderate antioxidant profile and agronomic traits. This group had low potassium and dietary fiber contents and thin stems. Cluster II consisted of a single genotype (Accession number 40) with high zinc, beta-carotene, vitamin C, TPC, calcium, protein, and dietary fiber, broad leaves and high biological yield. In contrast, the cluster II genotype had low iron, manganese, copper, TAC, potassium, and magnesium contents and a low shoot: root ratio. Cluster III contained six genotypes, enriched with magnesium and several antioxidants including iron, manganese, zinc, beta-carotene, TAC, TPC, and TFC. Genotypes in cluster III exhibited low copper, vitamin C, protein, and dietary fiber contents and had small and limited leaves with thin stems. Cluster IV was composed of 12 genotypes that had high manganese, copper, beta-carotene and protein contents but low iron, TFC, potassium, and dietary fiber contents with short stature and thin plants. Cluster IV genotypes also produced the lowest shoot weight, foliage, and biological yield among all of the clusters. Cluster V, which was composed of two genotypes (accession number 20 and 21), exhibited the highest iron and beta-carotene contents and high TAC, calcium, magnesium and protein contents. Cluster V genotypes also

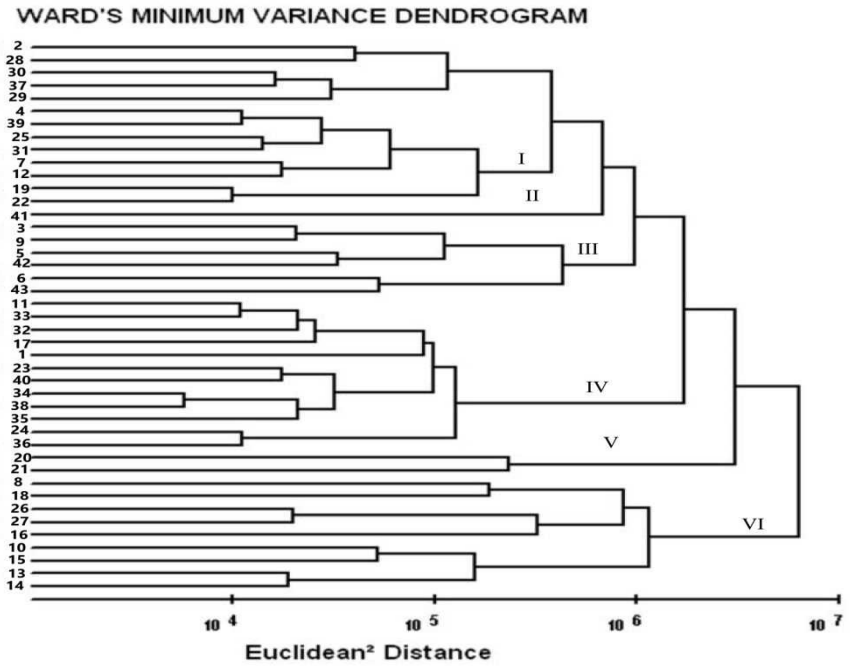

Fig. 1. Dendrogram of 43 vegetable amaranth genotypes using Ward's method.

had the lowest zinc, TPC, plant height, leaves plant^{-1}, and stem base diameter. Cluster VI consisted of nine genotypes, which were observed to be the best among all of the clusters for all of the antioxidant, nutrient and agronomic traits except beta-carotene, calcium, protein, and dietary fiber.

Table 2. Cluster means for antioxidant, nutrient, dietary fiber and agronomic traits in 43 vegetable amaranth genotypes.

	Traits	Cluster means					
		I	II	III	IV	V	VI
Antioxidant profile	Fe (µg g^{-1})	982.76	882.28	1422.12	934.90	2301.63	1165.85
	Mn (µg g^{-1})	232.08	176.49	288.88	264.54	221.32	244.81
	Cu (µg g^{-1})	28.32	20.09	20.86	24.58	21.39	27.87
	Zn (µg g^{-1})	975.73	1020.62	1022.75	925.60	806.33	1049.57
	Beta carotene (mg kg^{-1})	923.12	1242.24	757.52	1053.21	1015.48	618.37
	Vitamin C (mg kg^{-1})	759.49	1047.53	667.22	846.83	751.62	885.59
	TAC (TEAC mg kg^{-1}dw)	16.94	13.35	21.24	13.77	22.78	20.60
	TPC (GAE mg kg^{-1}dw)	15.64	16.32	16.50	15.43	14.88	16.40
	TFC (RE mg kg^{-1}dw)	100.75	105.64	118.88	97.80	105.57	120.35
Nutrient traits	K (g 100 g^{-1})	1.00	1.00	1.10	0.98	1.00	1.01
	Ca (g 100 g^{-1})	2.74	2.62	2.20	2.48	2.71	2.18
	Mg (g 100 g^{-1})	2.99	2.91	3.05	2.95	3.05	2.96
	Protein (g 100 g^{-1})	1.24	1.36	1.16	1.35	1.35	1.20
	Dietary fiber (g 100 g^{-1})	7.88	9.15	7.82	7.85	7.81	7.51
Agronomic traits	Plant height (cm)	27.00	30.31	28.26	26.31	24.43	41.35
	Leaves plant^{-1}	10.14	11.01	9.27	10.50	9.29	14.63
	Leaf area (cm^2)	118.35	234.54	88.50	145.77	96.07	206.43
	Shoot weight (g)	16.95	13.14	15.10	11.70	12.96	24.53
	Shoot: root ratio	11.11	9.88	14.91	12.36	15.10	24.79
	Stem base diameter (cm)	5.97	6.98	5.26	5.41	4.70	7.71
	Foliage yield (g)	505.27	560.56	462.80	358.03	395.65	744.88
	Biological yield m^{-2} (g)	1512.86	1552.88	1363.44	1066.54	1182.25	2212.71

The first two PCs were plotted to observe relationships between the clusters (Fig. 2). Clusters II, III, V, and VI have a clear separation on the biplot. But cluster I and cluster IV, having the

highest number of genotypes, were not clearly separated on the biplot. The single genotype of cluster II had a high positive coefficient of PC_1 and a negative coefficient of PC_2 and is plotted in the extreme lower left corner of the biplot. The genotypes of cluster VI (Accession 8 and 15) had positive coefficients of PC_1 and low and negative coefficients of PC_2 and thus occupied the extreme upper left corner of the biplot. The rest of the genotypes from the other clusters had positive coefficients of both the components. Accessions 16, 26 and 27 of cluster VI had high positive coefficients of both PCs and thus occupied the extreme upper right corner of the biplot. Accessions 20 and 21 had low positive coefficients of PC_1 but high positive coefficients of PC_2 and thus occupied the extreme lower right corner of the biplot.

With few exceptions, collections from the northeastern regions of Bangladesh were clearly grouped into cluster IV. Such strong relationship among diversity and geographical origin has been previously reported in amaranth [63]. Conversely, studies of oat [139] maize [140], bambara groundnut [158], and Ethiopian mustard [59] observed that genetic diversity did not follow the geographical diversity that supported the distribution of the genotypes of the rest of the clusters (clusters I, II, III, V and VI) in our study. The outcome of this analysis was consistent with the results obtained through PCA, with the major differences between the clusters attributed to the same traits that contributed the most to PC1 and PC2. Cluster VI consisted of nine genotypes of different eco-geographical regions, and among them, only three members (Accession number 16, 26 and 27) could be considered sources of genes for foliage and yield and related agronomic traits, as well as Mg and antioxidant profiles. Similarly, cluster I included 13 genotypes from different regions of Bangladesh, which were enriched with manganese, copper, calcium, and magnesium; had a moderate antioxidant profile and agronomic traits; and had a high biological yield. Moreover, cluster II consisted of a single genotype (Accession number 41) having high zinc, beta-carotene, vitamin C, TPC, calcium, protein, and dietary fiber contents; broader leaves; and high biological yield. Genotypes of these clusters might be considered sources of genes for the above-mentioned traits. In contrast, cluster III contained six genotypes from six different regions of Bangladesh and was enriched with magnesium and several antioxidants such as iron, manganese, zinc, beta-carotene, TAC, TPC, and TFC (except copper and vitamin C). Cluster IV was composed of 12 genotypes from different eco-geographical regions of Bangladesh and had high manganese, copper, beta-carotene and protein contents. Cluster V, comprising two genotypes from two different regions of Bangladesh (Accession number 20 and 21), exhibited the highest manganese, TAC, and

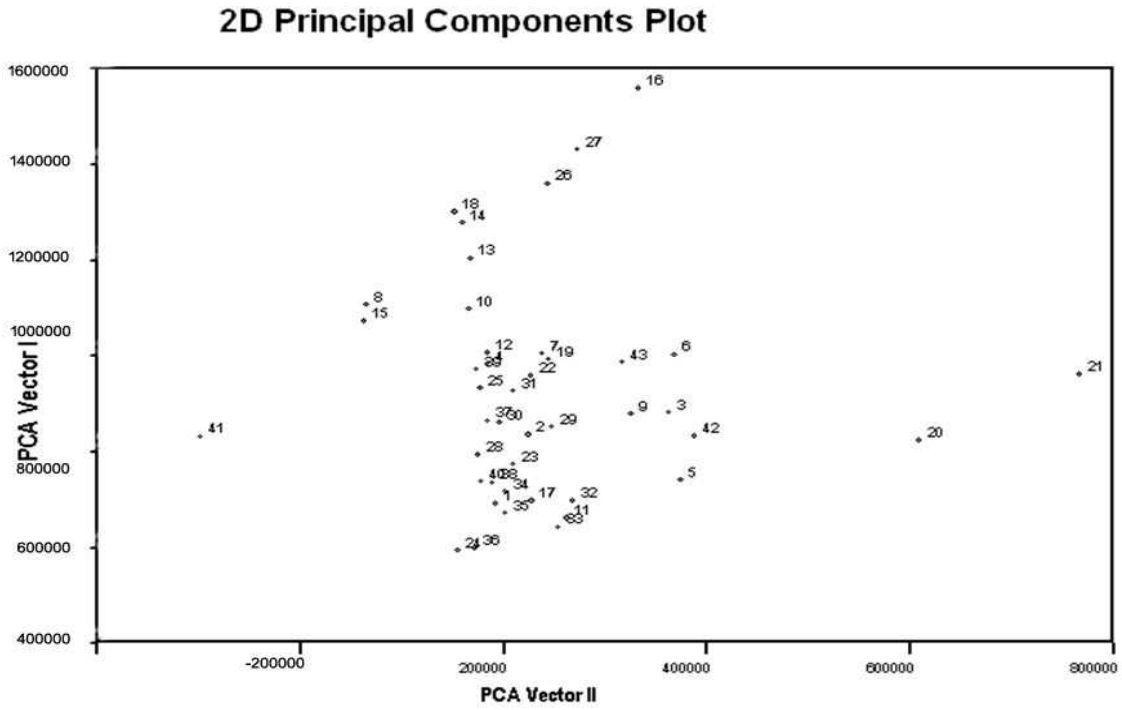

Fig. 2. Plot of the first and second component score for 43 vegetable amaranth genotypes.

magnesium contents and high beta-carotene, calcium and protein contents. Clusters III, IV and V might be considered donor parents for these traits. The absence of a relationship between genetic diversity and geographical diversity for clusters I, II, III, V and VI indicates that forces other than geographical origin, such as an exchange of genetic stocks, genetic drift, spontaneous variation, and natural and artificial selection, are perhaps responsible for the observed genetic diversity. Pandey [61] and Pandey and Singh [62] found similar trends in genetic and geographical diversity in grain amaranth. Our findings are in accordance with earlier reports that both PCA and cluster analysis can disclose complex relationships between taxa in a more understandable way and with equal effectiveness [157, 159].

Conclusively, high-yielding genotypes from cluster VI could be directly used as high antioxidant profile varieties. In contrast, low-yielding genotypes having desirable genes (any clusters) for a specific trait could be used as a source of donor parents in hybridization programs. Genotypes with desirable genes of one cluster hybridized with promising genotypes of other diverge clusters could facilitate the accumulation of favorable genes in hybrids.

Abstract

A lot of variations in vegetable amaranth germplasm have been observed in Bangladesh. It has been used as a cheap source of antioxidants, nutrients, protein, and dietary fiber. But no efforts had not been taken to know the status of antioxidant content, polyphenol, flavonoid, antioxidant

vitamins and minerals, dietary fiber, nutritional and agronomic traits. In this study, forty-three vegetable amaranth genotypes were evaluated to determine the status of total antioxidant content, polyphenol, flavonoid, antioxidant vitamins and minerals, dietary fiber, nutritional and agronomic traits and the magnitude of genetic diversity based on the contribution of those traits for meaningful grouping and proper utilization in future breeding program. The experiment was carried out in an open experimental field at Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh in a randomized complete block design with three replications. Multivariate (Principal component and cluster) analysis was done using numerical taxonomic techniques of Sneath, & Sokal. Four principal components contributed 98.61% of the variation. Biological yield and total antioxidant content were strongly associated with their related all agronomic traits. Total flavonoid content had a higher contribution to total antioxidant capacity compared to vitamin and mineral antioxidants. Contribution of antioxidant profile and agronomic traits was the highest in diversity of vegetable amaranth. Both high and low yielding genotypes had a high antioxidant profile. Therefore, high yielding genotypes (From cluster VI) could be used directly as high antioxidant profile varieties and low yielding genotypes as a source of donor parents in hybridization program. Cluster analysis grouped the genotypes into six clusters. The diverse genotypes in different clusters were identified. Genotypes with desirable genes of one cluster hybridized with promising genotypes of other diverge clusters could facilitate the accumulation of favorable genes in hybrids.

CHAPTER 3

ABIOTIC STRESS TOLERANCE OF VEGETABLE AMARANTH

3.1 Biochemistry and Food Aspect on Drought Stress of Vegetable Amaranth

Natural antioxidants, in vegetables, have gained the attention of both food researchers and consumers. Vegetable amaranth is a good source of minerals, vitamins, phenolics, and carotenoids; it also contains betalain, a nitrogen containing group of natural pigments, as well as proteins and fibers [6, 35]. Those secondary metabolites or natural antioxidants are involved in defenses against several diseases like cancer, atherosclerosis, arthritis, cataracts, emphysema, and retinopathy, neuro-degenerative and cardiovascular diseases [35, 50].

3.1.1 Response of nutrients, minerals, antioxidant leaf pigments, vitamins, polyphenol, flavonoid and antioxidant activity in selected vegetable amaranth under four soil water content

Purpose of the study

Amaranths are often described as drought tolerant plants [68, 69]. *Amaranthus tricolor* is a versatile food crop exhibiting high adaptability to new environments, even in the presence of different biotic and abiotic stresses [70]. The amount of metabolites in plants might be affected by different factors such as biological, environmental, biochemical, physiological, ecological, and evolutionary processes [71]. Among these factors, drought stress can highly enhance the concentration of secondary metabolites [72].

The degree of damage by reactive oxygen species (ROS) is highly related to the balance between ROS production and its removal by the antioxidant scavenging system [64]. On the other hand, it has been reported that the plant cell membrane was more sensitive to rapid damage and leakage under water stress [64]. Plants can synthesize some secondary metabolites i. e., α-tocopherol (vitamin E), and polyphenol to protect them against oxidative damage caused by environmental stresses [65, 66]. These compounds evolve to detoxify reactive oxygen species in plants, but they also show beneficial activity against some human diseases related to oxidative damage and aging [67].

There are few reports related to the effect of water stress on secondary metabolites of different crops including leafy vegetables. To date, scarce information is available for betalainic food crops under water stress, although betaxanthin and betacyanin have recently attracted attention for their antioxidant activities [73]. Water stress elevated secondary metabolites such as beta-carotene content in Choysum in dry season trial [74], in perennial

herbaceous [75], ascorbic acid in tomato [73], TPC, TFC in buckwheat [76], TPC, TFC and antioxidant activity in *Achillea* species [77]. In contrast, water stress reduced the protein content in buckwheat [76], beta-carotene content in Kailaan in dry season trial [77], ascorbic acid, Ca, Fe and Zn content [74]. In our previous studies [143, 149-151, 160-162] we selected some genotypes with a high content in antioxidants and high yield potential. Therefore, to fill the lacuna, present investigation aimed to study the selected vegetable amaranth genotypes in response to soil water stress in terms of proximate, minerals, betacyanin, betaxanthin, beta-carotene, ascorbic acid, TPC and TFC, and total antioxidant activity in.

Materials and methods

Experimental site, Plant materials, experiment design, layout

The experiment was conducted at the experimental field of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. Four genotypes with a high content in antioxidants and high yield potential from 102 genotypes collected in different echo-geographical regions of Bangladesh were selected on the basis of our earlier studies [143, 149-151, 160-162]. Accession numbers of the four genotypes were VA6, VA11, VA14 and VA16. These genotypes were sown in a randomized complete block design (RCBD) with three replications.

Imposing of water stress

The seedlings were raised in plastic pots of 22 cm height and 24 cm diameter (upper side). A homogenous mixture of soil and cow dung (2:1 ratio) was placed in the pots leaving few cm empty space at the top to hold the irrigation water. Pots were well irrigated up to 10 days after germination of seeds for proper establishment and vigorous growth of seedlings. After 10 days of seedlings establishment, they were subjected to four treatments of irrigation including 30% field capacity (FC) or severe water stress (SWS), 60% FC or moderate water stress (MWS), 90% FC or low water stress (LWS), and 100% FC (control). Leaf samples were collected from 30 days old plants of each experimental unit.

Chemicals

Solvent: methanol and acetone. Reagents: ascorbic acid, gallic acid, rutin, methanol, DPPH (2, 2-diphenyl1-picryl-hydrazyl), $ABTS^{+}$, trolox (6-hydroxy-2, 5, 7, 8-tetra-methyl-chroman-2-carboxylicacid), aluminum chloride hexa-hydrate, sodium carbonate, potassium acetate, Folin-Ciocalteu reagent, H_2SO_4, NaOH, HNO_3, $HClo_4$, lanthamum, Caesium chloride, dithiothreitol (DTT) and potassium persulfate. All solvents and reagents used in this study were

high purity laboratory products obtained from Kanto Chemical Co. Inc. (Tokyo, Japan) and Merck (Germany).

Proximate composition

Moisture content was measured following ASAE standards [163]. Briefly, triplicates of vegetable amaranth leaf samples were oven-dried at 103 °C for 72 h, transferred to a desiccator, and allowed to cool at room temperature. The sample weights were recorded on a digital balance (Denver Instruments, Denver, Colorado, USA).

Ash, crude fat, and crude protein contents were determined by AOAC methods (AOAC (Association of Analytical Chemists) [164]. Ash content was determined by weighing leaf samples before and after heat treatment (550 °C for 12 h). Crude fat content was determined according to AOAC method 960.39.

Crude protein was assessed by the micro-Kjeldahl method, with nitrogen to protein conversion factor of 6.25 (AOAC method 976.05). Fiber was determined by ISO method 5498 [165]. First, a sample of leaf powder was boiled in 0.255 M sulfuric acid for 30 min. The resulting insoluble residue was filtered, washed, and boiled in 0.313 M sodium hydroxide. After filtering and washing the sample, it was dried at 130 ± 2 °C for 2 h. Weight loss was determined at 350 ± 25 °C. Fiber content was expressed relative to the fresh weight (FW).

Carbohydrate content (g 100 g^{-1} FW) was calculated by subtracting the sum of percent moisture, ash, crude fat, and crude protein from 100. Gross energy was determined using a bomb calorimeter according to ISO method 9831 ([166].

Determination of mineral content

Leaves of vegetable amaranth were dried at 70 °C in a well-ventilated drying oven for 24 hours. Dried leaf of vegetable amaranth was ground finely in a mill. Milled powder was passed through an 841 microns screen. A portion of the dried power was analyzed for macronutrients (Ca, Mg, K, P and S) and microelements (Fe, Mn, Cu, Zn, Na, Mo and B). All macronutrients and microelements were extracted after dissolution of the vegetable amaranth samples by nitric-perchloric acid digestion [167]. According to Zasoski and Burau [168] nitric-perchloric acid digestion was performed by adding 0.5 g of the dried samples to 400 ml of nitric acid (65%) with 40 ml of perchloric acid (70%) and 10 ml of sulphuric acid (96%) in the presence of carborundum beads. After nitric-perchloric acid digestion, the solution was appropriately diluted and P analysis was performed in triplicate according to the ascorbic acid method [169]. In acidic medium, orthophosphates formed a yellow-colored complex with molybdate ions and, after addition of ascorbic acid and Sb, a blue-colored

phosphomolybdenum complex was formed. Absorbance was measured according to the method described by Temminghoff and Houba [170] in triplicate at wavelength 880 nm (P), 766.5 nm (K), 422.7 nm (Ca), 285.2 nm (Mg), 258.056 nm (S), 248.3 nm (Fe), 279.5 nm (Mn), 324.8 nm (Cu), 213.9 nm (Zn), 589.0 nm (Na), 313.3 nm (Mo) and 430 nm (B), by atomic absorption spectrophotometry (AAS) (Hitachi, Tokyo, Japan). For calibration, AAS standard solutions (1000 mg l^{-1} in 5% HNO_3) were purchased from Merck, Germany. Finally, interferences were controlled by the addition of lanthanum and caesium chloride (0.1%) to samples and standards.

Determination of betacyanin, betaxanthin, chlorophyll and beta-carotene content

betacyanin, betaxanthin, chlorophyll and beta-carotene were measured following the procedure described in the previous chapter

Ascorbic acid

The total ascorbic acid defined as ascorbic acid (AsA) and dehydroascorbate (DHA) acid was assessed by spectrophotometric detection on fresh plant tissues. The assay is based on the reduction of Fe_3^+ to Fe_2^+ by AsA and the spectrophotometric (Hitachi, U-1800, Tokyo, Japan) detection of Fe_2^+ complexes with 2, 2-dipyridyl [171]. DHA is reduced to AsA by pre-incubation of the sample with dithiothreitol (DTT). The absorbance of the solution was measured spectrophotometrically using a Hitachi U1800 instrument (Hitachi, Tokyo, Japan). Data were expressed as mg ascorbic acid per 100 g fresh weight.

Extraction samples for TPC, TFC and TAC analysis

Samples were extracted following the procedure described in the previous chapter

Determination of TPC, TFC and TAC

TPC, TFC and TAC were measured following the procedure described in the previous chapter

Statistical Analysis

The results were reported as the mean ± SD of six separate measurements (n = 6). The data were also statistically analyzed by ANOVA using Statistix 8 software, and the means were compared by the Duncan's multiple range (DMRT) test at 1% level of probability.

Results and discussion

Proximate composition

The proximate compositions were significantly affected by vegetable amaranth variety, soil water content and variety × soil water content interactions and presented in Table 1. Like other

leafy vegetables, our study showed that vegetable amaranth leaves are a good source of moisture, protein, dietary fiber and carbohydrates.

Table 1. Effect of soil water content on proximate composition (per 100 g fresh weight) in four selected vegetable amaranth genotypes

Treatment	Moisture (g)	Protein (g)	Fat (g)	Dietary fiber (g)	Carbohydrates (g)	Energy (Kcal)	Ash (g)
Variety × SWC							
VA6 × Control	86.18 ± 0.82b	3.15 ± 0.02p	0.23 ± 0.03i	7.45 ± 0.09m	7.42 ± 0.13c	42.43 ± 0.17h	3.03 ± 0.02n
VA6 × LWS	85.96 ± 0.75bc	3.27 ± 0.01o	0.22 ± 0.01j	7.65 ± 0.06l	7.14 ± 0.07c	42.28 ±0.18h	3.12 ± 0.04m
VA6 × MWS	85.30 ± 1.07e	4.27 ± 0.09k	0.19 ± 0.02k	8.22 ± 0.10h	6.96 ± 0.12c	44.84 ± 0.19g	3.36 ± 0.07j
VA6 × SWS	85.36 ± 1.15de	4.66 ± 0.04i	0.18 ± 0.01l	9.11 ± 0.08c	6.12 ± 0.15e	43.16 ± 0.11h	3.68 ± 0.11i
VA11 × Control	87.24 ± 0.69a	3.54 ± 0.06n	0.37 ± 0.03c	8.22 ± 0.04h	5.88 ± 0.05e	39.48 ± 0.21i	2.98 ± 0.09p
VA11 × LWS	86.25 ± 2.02b	3.65 ± 0.05m	0.36 ± 0.01d	8.78 ± 0.08f	6.76 ± 0.06d	43.09 ± 0.26h	2.99 ± 0.040
VA11 × MWS	86.12 ± 1.26b	3.73 ± 0.02l	0.33 ± 0.02g	9.37 ± 0.07b	6.66 ± 0.09d	42.83 ± 0.23h	3.13 ± 0.06l
VA11 × SWS	85.58 ± 0.88d	4.37 ± 0.10j	0.33 ± 0.01g	10.24 ± 0.11a	6.56 ± 0.07d	45.04 ± 0.19g	3.16 ± 0.03k
VA14 × Control	82.20 ± 0.19f	6.24 ± 0.07f	0.34 ± 0.03f	6.88 ± 0.06o	5.97 ± 0.14e	50.36 ± 0.16e	5.26 ± 0.07d
VA14 × LWS	82.27 ± 0.84f	6.35 ± 0.06e	0.35 ± 0.02e	7.21 ± 0.05n	5.69 ± 0.08f	49.80 ± 0.14f	5.35 ± 0.08c
VA14 × MWS	81.33 ± 0.23g	7.59 ± 0.03c	0.32 ± 0.01h	7.89 ± 0.12j	5.20 ± 0.04g	52.70 ± 0.25c	5.56 ± 0.09b
VA14 × SWS	81.29 ± 0.65g	8.26 ± 0.04a	0.32 ± 0.03h	8.88 ± 0.09e	4.25 ± 0.05g	51.83 ± 0.18d	5.88 ± 0.03a
VA16 × Control	81.35 ± 1.28g	5.39 ± 0.06h	0.41 ± 0.01a	7.85 ± 0.11k	8.39 ± 0.04a	56.68 ± 0.11b	4.46 ± 0.02h
VA16 × LWS	81.30 ± 0.88g	5.47 ± 0.05g	0.37 ± 0.02c	8.03 ± 0.13i	8.32 ± 0.07a	56.40 ± 0.21b	4.54 ± 0.06g
VA16 × MWS	80.25 ± 0.68h	6.98 ± 0.02d	0.37 ± 0.01c	8.68 ± 0.12g	7.80 ± 0.06b	60.48 ± 0.22a	4.60 ± 0.07f
VA16 × SWS	80.17 ± 1.14h	7.46 ± 0.07b	0.38 ± 0.01b	9.06 ± 0.07d	7.32 ± 0.07c	60.60 ± 0.27a	4.61 ± 0.08e
Variety							
VA6	85.70 ± 0.58b	3.84 ± 0.05c	0.21 ± 0.01c	8.11 ± 0.06c	6.91 ± 0.07b	43.18 ± 0.16c	3.30 ± 0.05c
VA11	86.30 ± 0.89a	3.83 ± 0.03c	0.35 ± 0.02b	9.15 ± 0.07a	6.46 ± 0.04c	42.61 ± 0.25c	3.06 ± 0.03d
VA14	81.77 ± 0.72c	7.11 ± 0.07a	0.34 ± 0.02b	7.72 ± 0.06d	5.28 ± 0.06d	51.17 ± 0.23b	5.51 ± 0.08a
VA16	80.77 ± 0.77d	6.33 ± 0.04b	0.38 ± 0.03a	8.41 ± 0.08b	7.96 ± 0.04a	58.54 ± 0.16a	4.55 ± 0.07b
SWC							
Control	84.24 ± 1.10a	4.58 ± 0.07d	0.34 ± 0.03a	7.60 ± 0.07d	6.91 ± 0.07a	47.24 ± 0.08b	3.93 ± 0.02d
LWS	83.94 ± 1.27a	4.69 ± 0.05c	0.33 ± 0.01a	7.92 ± 0.06c	6.98 ± 0.02a	47.89 ± 0.12b	4.00 ± 0.05c
MWS	83.25 ± 0.92b	5.64 ± 0.06b	0.31 ± 0.02b	8.54 ± 0.12b	6.66 ± 0.05a	50.21 ± 0.17a	4.16 ± 0.04b
SWS	83.10 ± 0.81b	6.19 ± 0.03a	0.30 ± 0.01b	9.32 ± 0.11a	6.06 ± 0.06b	50.16 ± 0.14a	4.33 ± 0.06a
Significance							
Variety	**	**	**	**	**	**	**
SWC	**	**	**	**	**	**	**
Variety × SWC	**	**	**	**	**	**	**

SWC = Soil water content, LWS = Low water stress, MWS = Medium water stress, SWS = Severe water stress, ** Significant at 1% level, (n = 6)
In a column, mean values with different letters are differed significantly by Duncan Multiple Range Test

Across varieties, VA16 exhibited the lowest moisture content (80.77 g 100 g^{-1}), the highest fat (0.38 g 100 g^{-1}), carbohydrates (7.96 g 100 g^{-1}), and energy (58.54 Kcal 100 g^{-1}) while, VA14 had low moisture, the highest protein (7.11 g 100 g^{-1}) and ash content (5.51 g 100 g^{-1}). The highest dietary fiber content was reported for genotype (or accession) VA11. VA16 showed 85% higher fat, 15% more carbohydrates and 36% higher energy compared to VA6. 85% higher protein and 67% more ash was recorded in VA14 over VA6 (Fig. 1 Supplementary file).

Considering soil water content, the highest moisture and fat content were observed in control and low water stress conditions, while the medium water stress (MWS) and severe water stress (SWS) exhibited the lowest moisture and fat content. The highest protein, dietary fiber and ash content were observed in SWS followed by MWS condition, while the control

had the lowest protein, dietary fiber and ash content followed by LWS. Protein, dietary fiber and ash content were significantly increased with the increment of soil water stress in the following order: control < LWS < MWS < SWS. In SWS, increase in protein and dietary fiber were 35% and 23% over control condition (Fig. 2, supplementary file). The highest carbohydrates content was observed in LWS (6.98 g 100 g^{-1}) which was statistically similar to control (6. 91 g 100 g^{-1}), and MWS (6. 66 g 100 g^{-1}). The lowest carbohydrates content was noticed in SWS (6.06 g 100 g^{-1}). Severity of soil water deficit resulted in reduction in carbohydrate content. Like other leafy vegetables, low carbohydrate content of *A. tricolor* does not have a significant impact on carbohydrate contribution in human body. The highest energy was observed in MWS (50.21 Kcal 100 g^{-1}) and SWS (50.16 Kcal 100 g^{-1}) condition, while the control and LWS exhibited the lowest energy (47.24 Kcal 100 g^{-1} and 47.89 Kcal 100 g^{-1}).

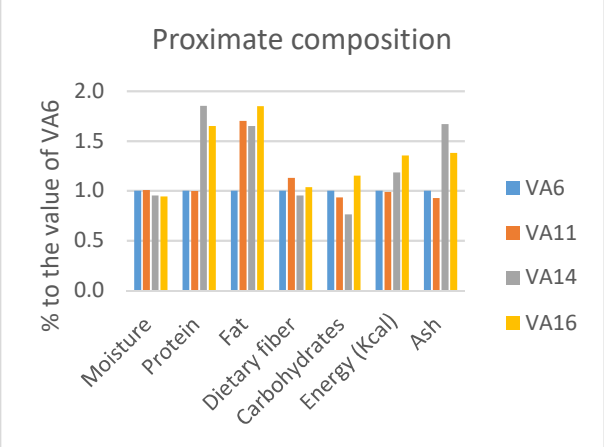

Fig. 1. Influence of proximate composition (g 100 g^{-1}) (% to the value of VA6) in selected *Amaranthus tricolor* genotypes

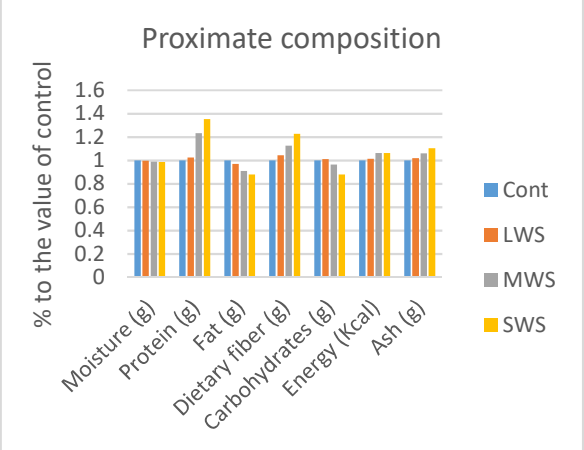

Fig. 2. Changes of proximate composition (g 100 g^{-1}) (% to the value of control) under four soil water content: Control (100% FC), LWS (90% FC), MWS (60% FC), and SWS (30% FC)

Regarding energy balance, both MWS and SWS had significantly higher values than control and LWS, although these differences do not have a significant impact on energy contribution in human body, since the low amounts consumed on a daily basis diet. With respect to variety × soil water content interaction, the highest moisture content was observed in VA11 under control (87.24 g 100 g^{-1}). In contrast, VA16 under MWS (80.25 g 100 g^{-1}) and VA16 under SWS (80.17 g 100 g^{-1}) had the lowest moisture content followed by VA16 under control, VA16 under LWS, VA14 under MWS and VA14 under SWS. The highest protein content was noticed in VA14 under SWS (8.26 g 100 g^{-1}) followed by VA14 under MWS (7.59 g 100 g^{-1}), VA16 under SWS (7.46 g 100 g^{-1}), VA16 under MWS (6.98 g 100 g^{-1}). In contrast, the lowest protein content was found in VA6 under control (3.15 g 100 g^{-1}) followed by VA6

under LWS (3.27 g 100 g^{-1}). In our study, protein content was significantly increased in the leaves of all the varieties in the following order: control < LWS < MWS < SWS. However, Siracusa *et al*. [76] observed reduction of protein content in full irrigated to water stress in buckwheat. This might be likely due to genotypic differences between to crops. The fat content ranged from 0.40 to 0.17 g 100 g^{-1}. The highest fat content was observed in VA16 under control (0.41 g 100 g^{-1}) followed by VA16 under SWS, VA16 under LWS, VA16 under MWS and VA11 under LWS. Conversely, VA6 under SWS (0.18 g 100 g^{-1}) had the lowest fat content. The highest fiber content was observed in VA11 under SWS (10.24 g 100 g^{-1}) followed by VA11 under MWS (9.37 g 100 g^{-1}), VA6 under SWS (9.11 g 100 g^{-1}), VA16 under SWS (9.09 g 100 g^{-1}). On the other hand, the lowest fiber content was observed in VA14 under control (6.88 g 100 g^{-1}) followed by VA14 under LWS (7.21 g 100 g^{-1}). Fiber content was significantly increased with the increment of soil water stress in all the varieties in following order: control < LWS < MWS < SWS. The highest carbohydrates content was observed in VA16 under control (8.39 g 100 g^{-1}) and VA16 under LWS (8.32 g 100 g^{-1}) followed by VA16 under MWS, VA16 under SWS, VA11 under LWS, VA11 under MWS and VA11 under SWS. Alternatively, VA14 under MWS (5.20 g 100 g^{-1}) and VA14 under SWS (4.25 g 100 g^{-1}) had the lowest carbohydrates content. Energy ranged from 42.27 to 60.59 Kcal. The highest energy was observed in VA16 under SWS (60.59 Kcal 100 g^{-1}) and VA16 under MWS (60.47 Kcal 100 g^{-1}) followed by VA16 under control, VA16 under SWS and VA14 under MWS. Instead, the lowest energy was observed in VA6 under control, VA6 under LWS, VA6 under SWS, VA11 under LWS and VA11 under MWS. The ash content ranged from 2.98 to 5.88 g 100 g^{-1}. The highest ash content was observed in VA14 under SWS (5.88 g 100 g^{-1}) followed by VA14 under MWS, VA14 under LWS and VA14 under control. VA11 under control (2.98 g 100 g^{-1}) showed the lowest ash content. Soil water stress increased the protein, dietary fiber, energy, ash content and decreased moisture, fat and carbohydrate content of vegetable amaranth. For this, amaranth produced in dry area and season could contribute as a good source of protein and fiber in human diet.

Mineral (macro and micro elements)

The mineral compositions of vegetable amaranth were significantly affected across variety, soil water content and variety × soil water content interactions and presented in Table 2 and Table 3. Within varieties, the highest Ca, K, P, S, Mn, Cu, Zn, Na and B content were observed in VA14 and the highest Mg, Fe, Zn and Mo were recorded in VA16. Whereas, VA6 exhibited the lowest Fe, Mn, Cu and Mo and VA11 showed the lowest K, P, S, Na and B content. VA14 had 50%, 34%, 114%, 68%, 51%, 121%, 15%, 12% and 47% increase in Ca, K, P, S, Mn, Cu,

Zn, Na and B, respectively over VA6 and VA16 exhibited 16%, 50%, 13% and 171% increase in Mg, Fe, Zn and Mo content respectively, over VA6 (Fig. 3 Supplementary file). These results were fully agreed with the results of Hanson *et al*. [74] where they found varietal differences in mineral content of Kailaan and Choysum.

Table 2. Effect of soil water content on mineral (macro-elements mg/g FW) composition in four selected vegetable amaranth genotypes

Treatment	Ca	Mg	K	P	S
Variety × SWC					
VA6 × Control	2.26 ± 0.06j	3.59 ± 0.02i	5.92 ± 0.01l	0.78 ± 0.02g	0.77 ± 0.02k
VA6 × LWS	2.58 ± 0.10i	3.60 ± 0.01h	6.07 ± 0.02j	0.68 ± 0.01h	0.64 ± 0.01m
VA6 × MWS	3.50 ± 0.05f	4.45 ± 0.03e	6.66 ± 0.02i	0.65 ± 0.02i	1.25 ± 0.02h
VA6 × SWS	4.47 ± 0.02d	4.72 ± 0.06c	7.25 ± 0.04h	0.51 ± 0.03l	1.53 ± 0.01f
VA11 × Control	2.58 ± 0.01i	3.15 ± 0.02m	4.66 ± 0.02o	0.65 ± 0.02i	0.51 ± 0.02o
VA11 × LWS	2.75 ± 0.06h	3.06 ± 0.05n	4.98 ± 0.01n	0.62 ± 0.01j	0.55 ± 0.03n
VA11 × MWS	3.70 ± 0.04e	3.42 ± 0.01j	7.32 ± 0.05h	0.55 ± 0.05k	0.71 ± 0.02l
VA11 × SWS	5.05 ± 0.01c	3.91 ± 0.02g	8.11 ± 0.02d	0.47 ± 0.02m	1.08 ± 0.01i
VA14 × Control	3.25 ± 0.02g	2.49 ± 0.01p	7.54 ± 0.07f	1.75 ± 0.04a	1.27 ± 0.02g
VA14 × LWS	3.72 ± 0.07e	2.86 ± 0.04o	7.96 ± 0.06e	1.72 ± 0.02b	1.68 ± 0.04d
VA14 × MWS	5.54 ± 0.02b	3.35 ± 0.07k	8.86 ± 0.08c	1.05 ± 0.01d	1.85 ± 0.02c
VA14 × SWS	6.66 ± 0.01a	4.54 ± 0.06d	10.39 ± 0.02b	1.11 ± 0.02c	2.22 ± 0.01a
VA16 × Control	1.68 ± 0.02m	3.21 ± 0.01l	5.64 ± 0.03m	1.11 ± 0.02c	1.07 ± 0.02i
VA16 × LWS	1.85 ± 0.03l	3.96 ± 0.02f	5.98 ± 0.02k	1.06 ± 0.01d	1.03 ± 0.04j
VA16 × MWS	2.14 ± 0.04k	5.46 ± 0.02b	7.36 ± 0.03g	0.98 ± 0.04e	1.57 ± 0.03e
VA16 × SWS	2.56 ± 0.02i	6.28 ± 0.02a	11.46 ± 0.03a	0.95 ± 0.02f	1.97 ± 0.01b
Variety					
VA6	3.20 ± 0.03c	4.09 ± 0.03b	6.47 ± 0.05c	0.66 ± 0.01c	1.05 ± 0.02c
VA11	3.52 ± 0.05b	3.39 ± 0.06c	6.27 ± 0.04d	0.57 ± 0.02d	0.71 ± 0.03d
VA14	4.79 ± 0.02a	3.31 ± 0.02d	8.69 ± 0.04a	1.41 ± 0.03a	1.76 ± 0.01a
VA16	2.06 ± 0.01d	4.73 ± 0.05a	7.61 ± 0.02b	1.03 ± 0.02b	1.41 ± 0.02b
SWC					
Control	2.44 ± 0.02d	3.11 ± 0.04d	5.94 ± 0.06d	1.07 ± 0.03a	0.91 ± 0.02d
LWS	2.73 ± 0.05c	3.37 ± 0.02c	6.25 ± 0.04c	1.02 ± 0.03b	0.97 ± 0.01c
MWS	3.72 ± 0.02b	4.17 ± 0.05b	7.55 ± 0.03b	0.81 ± 0.02c	1.34 ± 0.03b
SWS	4.68 ± 0.01a	4.86 ± 0.03a	9.30 ± 0.04a	0.76 ± 0.01d	1.70 ± 0.04a
Significance					
Variety	**	**	**	**	**
SWC	**	**	**	**	**
Variety × SWC	**	**	**	**	**

SWC = Soil water content, LWS = Low water stress, MWS = Medium water stress, SWS = Severe water stress, ** Significant at 1% level, (n = 6)
In a column, mean values with different letters are differed significantly by Duncan Multiple Range Test

Across soil water content, Ca, Mg, K, S, Mn, Cu, Na, Mo and B content were significantly increased with the increment of soil water stress in the following order: control < LWS < MWS < SWS. In SWS, the rate of increment of Ca, S, Mn, Cu, Mg, K, Mo, B and Na was 92%, 87%, 85%, 75, 56%, 57%, 45%, 36%, and 26% respectively, over control (Fig. 4 supplementary file). In contrast, Hanson *et al*. [74] reported decreasing trend in Ca content both in Choysum and Kailaan varieties from wet season to dry season trial. Further, it was noted that, increasing soil water stress lead to a significant decrease in Fe, P and Zn content in the following order: control

> LWS > MWS > SWS. In SWS, reduction of Fe, P and Zn were 58%, 29% and 19% respectively, over control. (Fig. 4 supplementary file). The highest Ca, Mg, K, S, Mn, Cu, Na, Mo and B content were observed in SWS while, the lowest Ca, Mg, K, S, Mn, Cu, Na, Mo and B content were found in control. Conversely, the highest P, Fe and Zn content were recorded in control and the lowest P, Fe and Zn content were found in SWS.

Table 3. Response of soil water content on mineral composition (micro-elements µg/g FW) in four selected vegetable amaranth genotypes

Treatment	Fe	Mn	Cu	Zn	Na	Mo	B
Variety × SWC							
VA6 × Control	12.99 ± 0.12h	12.25 ± 0.09p	1.27 ± 0.02n	11.33 ± 0.14h	72.24 ± 0.76m	0.26 ± 0.01n	5.52 ± 0.07n
VA6 × LWS	11.56 ± 0.09j	13.88 ± 0.07m	1.27 ± 0.03n	11.07 ± 0.21i	74.37 ± 0.87l	0.27 ± 0.02m	5.66 ± 0.08m
VA6 × MWS	8.35 ± 0.11m	16.88 ± 0.08i	1.55 ± 0.05l	10.54 ± 0.09m	82.45 ± 1.12g	0.28 ± 0.01l	5.78 ± 0.11l
VA6 × SWS	5.43 ± 0.02o	20.35 ± 0.11e	1.82 ± 0.02j	9.83 ± 0.24n	91.33 ± 1.22d	0.32 ± 0.03j	6.65 ± 0.08h
VA11 × Control	15.45 ± 0.06d	12.86 ± 0.21o	1.27 ± 0.01n	12.19 ± 0.22e	72.34 ± 1.26m	0.28 ± 0.01l	5.27 ± 0.06p
VA11 × LWS	14.25 ± 0.07f	12.94 ± 0.09n	1.31 ± 0.04m	12.12 ± 0.26f	72.37 ± 0.68m	0.28 ± 0.02l	5.33 ± 0.05o
VA11 × MWS	10.77 ± 0.04k	14.85 ± 0.11l	1.77 ± 0.03k	11.33 ± 0.25h	78.97 ± 1.24j	0.31 ± 0.01k	5.87 ± 0.08k
VA11 × SWS	7.78 ± 0.06n	18.95 ± 0.14f	1.97 ± 0.02i	10.89 ± 0.18j	88.92 ± 0.85e	0.36 ± 0.01i	6.28 ± 0.12j
VA14 × Control	16.72 ± 0.05b	16.77 ± 0.26j	2.26 ± 0.01g	14.61 ± 0.27a	80.28 ± 0.89i	0.56 ± 0.02h	7.36 ± 0.11f
VA14 × LWS	15.26 ± 0.04e	17.90 ± 0.16g	2.89 ± 0.04e	14.25 ± 0.17b	85.69 ± 1.17f	0.59 ± 0.01f	7.78 ± 0.14e
VA14 × MWS	12.77 ± 0.05i	26.73 ± 0.16c	3.77 ± 0.06c	10.58 ± 0.23l	92.34 ± 1.15c	0.64 ± 0.04d	9.27 ± 0.09c
VA14 × SWS	9.64 ± 0.03l	34.25 ± 0.13a	4.17 ± 0.04a	9.83 ± 0.27n	100.39 ± 1.05b	0.72 ± 0.02c	10.23 ± 0.06a
VA16 × Control	17.35 ± 0.02a	15.35 ± 0.21k	2.05 ± 0.03h	12.95 ± 0.25c	78.64 ± 1.18k	0.57 ± 0.01g	6.29 ± 0.08i
VA16 × LWS	16.69 ± 0.06c	17.57 ± 0.15h	2.46 ± 0.05f	12.89 ± 0.26d	80.87 ± 1.28h	0.62 ± 0.02e	6.75 ± 0.14g
VA16 × MWS	13.57 ± 0.05g	24.84 ± 0.18d	3.66 ± 0.05d	11.76 ± 0.11g	88.78 ± 1.09e	0.79 ± 0.03b	8.28 ± 0.11d
VA16 × SWS	9.65 ± 0.03l	32.11 ± 0.24b	4.01 ± 0.06b	10.66 ± 0.26k	102.31 ± 1.15a	1.05 ± 0.01a	10.15 ± 0.07b
Variety							
VA6	9.58 ± 0.03d	15.84 ± 0.08d	1.48 ± 0.02d	10.69 ± 0.13c	80.10 ± 1.04c	0.28 ± 0.02d	5.90 ± 0.06c
VA11	12.06 ± 0.04c	14.90 ± 0.11c	1.58 ± 0.03c	11.63 ± 0.16b	78.15 ± 1.15d	0.31 ± 0.02c	5.69 ± 0.08d
VA14	13.60 ± 0.06b	23.91 ± 0.12b	3.27 ± 0.02a	12.32 ± 0.21a	89.68 ± 0.99a	0.63 ± 0.01b	8.66 ± 0.04a
VA16	14.32 ± 0.04a	22.47 ± 0.15a	3.05 ± 0.04b	12.07 ± 0.25a	87.65 ± 0.87b	0.76 ± 0.03a	7.87 ± 0.11b
SWC							
Control	15.63 ± 0.02a	14.31 ± 0.16d	1.71 ± 0.03d	12.77 ± 0.16a	75.88 ± 1.27d	0.42 ± 0.02d	6.11 ± 0.12d
LWS	14.44 ± 0.05b	15.57 ± 0.18c	1.99 ± 0.03c	12.58 ± 0.19b	78.33 ± 1.18c	0.44 ± 0.01c	6.38 ± 0.08c
MWS	11.37 ± 0.03c	20.83 ± 0.16b	2.69 ± 0.04b	11.05 ± 0.24c	85.64 ± 1.32b	0.51 ± 0.02b	7.30 ± 0.05b
SWS	8.13 ± 0.05d	26.42 ± 0.18a	2.99 ± 0.05a	10.30 ± 0.22d	95.74 ± 1.28a	0.61 ± 0.02a	8.33 ± 0.09a
Significance							
Variety	**	**	**	**	**	**	**
SWC	**	**	**	**	**	**	**
Variety × SWC	**	**	**	**	**	**	**

SWC = Soil water content, LWS = Low water stress, MWS = Medium water stress, SWS = Severe water stress, ** Significant at 1% level, (n = 6)

In a column, mean values with different letters are differed significantly by Duncan Multiple Range Test

Similarly, Hanson *et al*. [74] found decreasing trend in Fe content in Choysum variety whereas, they found an increasing trend in Kailaan variety from wet season to dry season trial. However, Hanson *et al*. [74] found decreasing trend in Zn content both in Choysum and Kailaan variety from wet season to dry season trial. Among the interaction of variety × soil water content, Ca content ranged from 1.68 to 6.66 mg g^{-1} FW. The highest Ca content was observed in VA14 under SWS (6.66 mg g^{-1} FW) followed by VA14 under MWS, VA11 under SWS and VA6 under SWS. Instead, VA16 under control (1.68 mg g^{-1} FW) showed the lowest Ca content. Ca,

Mg, K, S, Mn, Cu, Na, Mo and B content were significantly increased with the increment of soil water stress in all the varieties in the following order: control < LWS < MWS < SWS. Mg content ranged from 2.49 to 6.28 mg g^{-1} FW. The highest Mg content was observed in VA16 under SWS (6.28 mg g^{-1} FW) followed by VA16 under MWS, VA6 under SWS and VA14 under SWS. In contrast, VA14 under control (2.49 mg g^{-1} FW) showed the lowest Mg content. K content ranged from 4.66 to 11.46 mg g^{-1} FW. The highest K content was observed in VA16 under SWS (11.46 mg g^{-1} FW) followed by VA14 under SWS, VA14 under MWS and VA11 under SWS. In contrast, VA11 under control (4.66 mg g^{-1} FW) showed the lowest K content. S content ranged from 0.51 to 2.22 mg g^{-1} FW. The highest S content was observed in VA14 under SWS (2.22 mg g^{-1} FW) followed by VA16 under SWS, VA14 under MWS, VA14 under LWS and VA16 under MWS. In contrast, VA11 under control (0.51 mg g^{-1} FW) showed the lowest S content. Mn content ranged from 12.86 to 34.25 µg g^{-1} FW. The highest Mn content was observed in VA14 under SWS (34.25 µg g^{-1} FW) followed by VA16 under SWS, VA14 under MWS, VA16 under MWS, VA6 under SWS and VA11 under control (12.86 µg g^{-1} FW). The highest Cu content was observed in VA14 under SWS (4.17 µg g^{-1} FW) followed by VA16 under SWS, VA14 under MWS and VA16 under MWS. In contrast, VA11 under control, VA6 under control and VA6 under LWS (1.27 µg g^{-1} FW) showed the lowest Cu content. Na content ranged from 72.24 to 102.31 µg g^{-1} FW. The highest Na content was observed in VA16 under SWS (102.31 µg g^{-1} FW) followed by VA14 under SWS, VA14 under MWS, VA6 under SWS and VA16 under MWS. VA6 under control (72.24 µg g^{-1} FW), VA11 under control (72.34 µg g^{-1} FW) and VA11 under SWS (72.37 µg g^{-1} FW) exhibited the lowest Na content. Mo content ranged from 0.26 to 1.05 µg g^{-1} FW. The highest Mo content was observed in VA16 under SWS (1.05 µg g^{-1} FW) followed by VA16 under MWS, VA14 under SWS, VA14 under MWS and VA16 under LWS. In contrast, VA6 under control (0.26 µg g^{-1} FW) exhibited the lowest Mo content. B content ranged from 5.27 to 10.23 µg g^{-1} FW. The highest B content was observed in VA14 under SWS (10.23 µg g^{-1} FW) followed by VA16 under SWS, VA14 under MWS, VA16 under MWS and VA14 under LWS. In contrast, VA11 under control (5.27 µg g^{-1} FW) exhibited the lowest B content. P content ranged from 0.47 to 1.07 mg g^{-1} FW. The highest P content was observed in VA14 under control (1.07 mg g^{-1} FW) followed by VA14 under LWS, VA14 under SWS, VA16 under control and VA14 under MWS. In contrast, VA11 under SWS (0.47 mg g^{-1} FW) showed the lowest P content. A significant decrement in P, Fe and Zn content were observed with the increase in soil water stress in all the varieties in the following order: control > LWS > MWS > SWS. The Fe content ranged from 5.43 to 17.35 µg g^{-1} FW. The highest Fe content was observed in VA16 under control (17.35 µg g^{-1} FW)

followed by VA14 under control, VA16 under LWS and VA11 under control. In contrast, VA6 under SWS (5.43 µg g^{-1} FW) showed the lowest Fe content. The highest Zn content was observed in VA14 under control (14.61 µg g^{-1} FW) followed by VA14 under LWS, VA16 under control and VA16 under LWS. In contrast, VA6 under SWS (9.83 µg g^{-1} FW) showed the lowest Zn content. All the macro and micro elements except P, Fe and Zn were increased with the severity of soil water stress, whereas, P, Fe and Zn were decreased with the severity of soil water deficit. For this, amaranth cultivated in stressful area especially in dry area and season could contribute as good source minerals in human diet compared to normal cultivation practices.

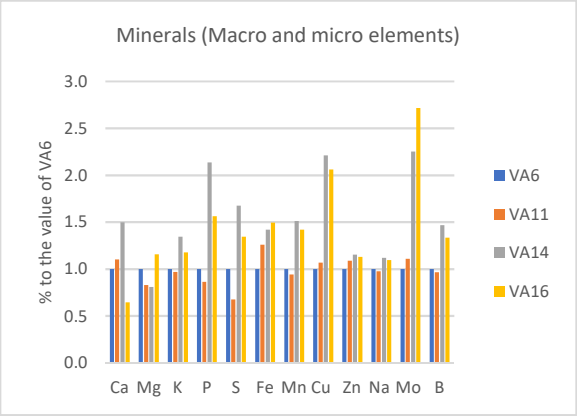

Fig. 3. Mineral (Macro mg g^{-1} and micro µg g^{-1} elements) contents (% to the value of VA6) in selected *Amaranthus tricolor* genotypes

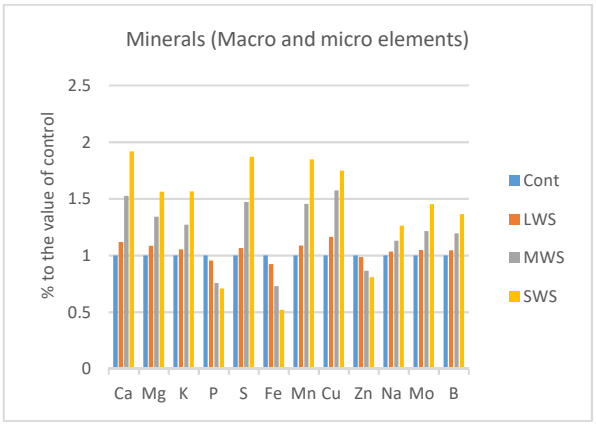

Fig. 4. Comparison of minerals (Macro mg g^{-1} and micro µg g^{-1} elements) (% to the value of control) under four soil water content: Control (100% FC), LWS (90% FC), MWS (60% FC), and SWS (30% FC)

Antioxidant leaf pigments, ascorbic acid content, TPC, TFC and TAC

Antioxidant leaf pigments, ascorbic acid content, TPC, TFC and TAC of vegetable amaranth were significantly affected by variety, soil water content and variety × soil water content interactions and presented in Table 4 and Table 5.

Considering varieties, the highest betacyanin, betaxanthin, betalain, beta-carotene, ascorbic acid, TPC and TFC was recorded in VA14 and the highest chlorophyll a, chlorophyll b and total chlorophyll, TAC (DPPH) and TAC ($ABTS^{+}$) was obtained from VA16. While, VA6 exhibited the lowest betacyanin, betaxanthin, betalain, chlorophyll a, chlorophyll b and total chlorophyll, ascorbic acid, TPC, TFC, TAC (DPPH) and TAC ($ABTS^{+}$) and VA16 had the lowest beta-carotene content. VA14 had 170%, 48%, 90%, 38%, 118%, 101% and 63% increase in betacyanin, betaxanthin, betalain, beta-carotene, ascorbic acid, TPC and TFC, respectively, compared to VA6. VA16 showed 2.6-fold chlorophyll a, chlorophyll b and total

chlorophyll and more than one-fold TAC (DPPH) and TAC ($ABTS^{+}$) increase compared to VA6 (Fig. 5 supplementary file). Hanson *et al*. [74] reported that beta-carotene and ascorbic acid content were differed significantly in Choysum and Kailaan varieties. Similar varietal differences were observed for beta-carotene in perennial herbaceous [75], ascorbic acid in tomato [73], TPC, TFC in buckwheat [76], TPC, TFC and antioxidant activity in *Achillea* species [77].

Table 4. Influence of soil water content on antioxidant leaf pigments in four selected vegetable amaranth genotypes

Genotype	betacyanin (ng g^{-1})	betaxanthin (ng g^{-1})	Betalain (ng g^{-1})	Chl a (µg g^{-1})	Chl b (µg g^{-1})	Total chl (µg g^{-1})	beta-carotene (mg g^{-1})
Variety × SWC							
VA6 × Control	185.02 ± 1.23m	355.98 ± 1.21k	542.35 ± 1.28m	156.09 ± 0.32m	64.90 ± 0.07m	221.62 ± 1.14m	0.82 ± 0.03i
VA6 × LWS	183.88 ± 0.89n	350.31 ± 1.08l	540.57 ± 0.88n	150.56 ±0.27n	64.86 ± 0.17m	218.57 ± 0.89n	0.83 ± 0.02i
VA6 × MWS	164.18 ± 0.47o	315.57 ± 0.82n	486.72 ± 1.18o	130.16 ± 0.32o	62.74 ± 0.21n	198.57 ± 0.74o	0.86 ± 0.04h
VA6 × SWS	160.02 ± 0.68p	295.97 ± 0.69p	462.45 ± 1.26p	80.31 ± 0.41p	54.49 ± 0.24o	139.59 ± 0.66p	0.98 ± 0.02f
VA11 × Control	378.97 ± 1.04g	372.48 ± 0.53i	752.44 ± 2.23h	443.63 ± 0,52g	221.52 ± 0.22f	666.75 ± 0.45g	0.70 ± 0.05m
VA11 × LWS	374.47 ± 2.01h	368.60 ± 0.82j	750.64 ± 1.47i	441.05 ± 0.28h	213.36 ±0.32g	658.69 ± 0.37h	0.72 ± 0.06l
VA11 × MWS	348.21 ± 1.26j	348.48 ± 0.67m	699.75 ± 0.97k	260.01 ± 0.36k	157.28 ± 0.33i	425.38 ± 0.95k	0.82 ± 0.04i
VA11 × SWS	340.02 ± 0.59k	308.89 ± 0.57o	654.70 ± 0.56l	230.95 ± 0.54l	115.27 ± 0.42l	346.50 ± 0.88l	1.29 ± 0.05b
VA14 × Control	499.01 ± 0.72a	501.10 ±0.44a	1000.46 ± 0.77a	515.04 ± 0.46a	251.46 ± 0.46d	768.47 ± 0.78a	1.01 ± 0.01e
VA14 × LWS	488.62 ± 2.12b	492.44 ± 0.88b	992.87 ± 0.26b	507.61 ± 0.72b	249.75 ± 0.71e	758.37 ± 0.53c	1.13 ± 0.02d
VA14 × MWS	462.13 ± 1.23e	488.23 ± 1.26d	958.27 ± 1.21d	490.72 ± 0.63e	187.18 ± 0.64h	737.63 ± 0.44f	1.26 ± 0.03c
VA14 × SWS	425.76 ± 0.94f	468.03 ± 1.32f	899.67 ± 1.44f	298.83 ± 0.65j	129.77 ± 0.55k	428.34 ± 0.58j	1.41 ± 0.02a
VA16 × Control	481.80 ± 0.58c	489.97 ± 1.14c	972.38 ± 1.15c	505.06 ± 0.43c	261.38 ± 0.67a	766.45 ± 0.53b	0.75 ± 0.04k
VA16 × LWS	475.54 ± 1.25d	481.65 ± 2.04e	956.67 ± 1.23e	452.82 ± 0.44f	258.46 ± 0.78b	755.68 ± 0.33d	0.77 ± 0.06j
VA16 × MWS	358.41 ± 1.22i	430.24 ± 1.55g	795.65 ± 1.46g	490.98 ± 0.51d	254.48 ± 0.69c	745.62 ± 0.89e	0.86 ± 0.02h
VA16 × SWS	328.47 ± 0.76l	412.60 ± 1.19h	744.55 ± 1.05j	392.72 ± 0.64i	135.67 ± 0.48j	528.67 ± 0.99i	0.92 ± 0.01g
Variety							
VA6	173.28 ± 0.35d	329.46 ± 0.65d	508.02 ± 0.48d	129.28 ± 0.18d	61.75 ± 0.25d	194.59 ± 0.36d	0.87 ±0.05c
VA11	360.42 ± 0.67c	349.61 ± 0.47c	714.38 ± 0.89c	343.91 ± 0.32c	176.86 ± 0.85c	524.33 ± 0.75c	0.88 ± 0.06b
VA14	468.88 ± 0.48a	487.45 ± 0.38a	962.82 ± 0.84a	453.05 ± 0.09b	204.54 ± 0.54b	673.20 ± 0.82b	1.20 ± 0.03a
VA16	411.05 ± 0.72b	453.61 ± 0.36b	867.31 ± 1.15b	460.40 ±0.23a	227.50 ± 0.57a	699.10 ± 0.87a	0.82 ± 0.03d
SWC							
Control	386.20 ± 0.46a	429.88 ± 0.52a	816.91 ± 1.24a	404.96 ± 0.34a	199.81 ± 0.08a	605.82 ± 0.48a	0.82 ± 0.01d
LWS	380.63 ± 0.53b	423.25 ± 0.46b	810.19 ± 1.07b	388.01 ± 0.46b	196.61 ± 0.24b	597.83 ± 0.41b	0.86 ± 0.02c
MWS	333.23 ± 0.61c	395.63 ± 0.25c	735.10 ± 0.86c	342.97 ± 0.55c	165.42 ± 0.17c	526.80 ± 0.52c	0.95 ± 0.04b
SWS	313.57 ± 0.65d	371.37 ± 0.22d	690.34 ± 0.58d	250.70 ± 0.48d	108.80 ± 0.12d	360.77 ± 0.62d	1.15 ± 0.02a
Significance							
Variety	**	**	**	**	**	**	**
SWC	**	**	**	**	**	**	**
Variety × SWC	**	**	**	**	**	**	**

SWC = Soil water content, LWS = Low water stress, MWS = Medium water stress, SWS = Severe water stress, ** Significant at 1% level, Chl a = chlorophyll a, Chl b = chlorophyll b, Total chl = Total chlorophyll, (n = 6)
In a column, mean values with different letters are differed significantly by Duncan Multiple Range Test

Among soil water content, betacyanin, betaxanthin, betalain, chlorophyll a, chlorophyll b and total chlorophyll content were significantly decreased with increasing soil water stress (control > LWS > MWS > SWS). In SWS, the reduction in betacyanin, betaxanthin, betalain, chlorophyll a, chlorophyll b and total chlorophyll content were 19%, 14%, 15%, 38%, 45% and 40%, respectively, compared to control (Fig 6 supplementary file). Similarly, Hsu and Kao [172] observed chlorophyll reduction with severity of water deficit in soil. They reported that

soil water deficit affected plant growth and development through osmotic stress on plants, reducing the water potential, decreasing stomatal conductivity which restricts CO_2 influx to leaves and unfavorable CO_2/O_2 ratio in chloroplasts, reducing photosynthesis.

However, in our study, we found that beta-carotene, ascorbic acid content, TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) were significantly increased with increasing soil water stress in the following order: control < LWS < MWS < SWS. In SWS, beta-carotene, ascorbic acid content, TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) were increased in 40%, 35%, 83%, 29%, 37% and 52% compared to control, respectively highest betacyanin, betaxanthin, betalain, chlorophyll a, chlorophyll b and total chlorophyll content were observed in control while, the lowest betacyanin, betaxanthin, betalain, chlorophyll a, chlorophyll b and total chlorophyll content were found in SWS. The highest beta-carotene, ascorbic acid, TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) were observed in SWS while, the lowest beta-carotene, ascorbic acid, TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) were observed in control.

Table 5. Effect of soil water stress on ascorbic acid content, TPC, TFC and TAC in four selected vegetable amaranth genotypes

Genotype	Ascorbic acid (mg 100 g^{-1})	Total polyphenol content (GAE µg g^{-1} dw)	Total flavonoid content (RE µg g^{-1} dw)	Total antioxidant capacity (DPPH) (TEAC µg g^{-1} dw)	Total antioxidant capacity (ABTS+) (TEAC µg g^{-1} dw)
Variety × SWC					
VA6 × Control	72.45 ± 0.15p	9.34 ± 0.04o	176.45 ± 1.02f	12.27 ± 0.080	26.69 ± 0.32p
VA6 × LWS	73.21 ± 0.22o	9.92 ± 0.07n	177.25 ± 0.87f	12.05 ± 0.14p	27.79 ± 0.43o
VA6 × MWS	88.43 ± 0.26n	11.78 ± 0.05m	180.66 ± 0.75e	15.16 ± 0.12n	34.99 ± 0.41n
VA6 × SWS	94.82 ± 0.16m	24.47 ± 0.02h	228.39 ± 0.67c	17.85 ± 0.07m	37.49 ± 0.54m
VA11 × Control	108.77 ± 0.18l	22.72 ± 0.07j	154.89 ± 0.55i	26.55 ± 0,14i	52.83 ± 0.28j
VA11 × LWS	110.27 ± 0.14k	23.63 ± 0.11i	160.63 ± 0.57h	27.25 ± 0.12h	56.79 ± 0.25h
VA11 × MWS	128.67 ± 0.18i	28.47 ± 0.09d	192.44 ± 0.58d	29.88 ± 0.21e	72.95 ± 0.22e
VA11 × SWS	142.47 ± 0.21g	38.41 ± 0.08a	220.42 ± 0.81c	33.88 ± 0.22b	83.34 ± 0.32a
VA14 × Control	156.34 ± 0.26e	25.55 ± 0.06g	280.44 ± 0.72b	24.38 ± 0.24l	48.79 ± 0.35l
VA14 × LWS	160.55 ± 0.19d	26.05 ± 0.06f	283.53 ± 0.58b	25.28 ± 0.18k	50.33 ± 0.36k
VA14 × MWS	188.57 ± 0.23b	28.55 ± 0.03d	335.86 ± 0.56a	27.56 ± 0.16f	56.99 ± 0.32g
VA14 × SWS	210.67 ± 0.26a	34.72 ± 0.05c	346.32 ± 0.52a	32.86 ± 0.28d	74.31 ± 0.28c
VA16 × Control	128.57 ± 0.16j	15.75 ± 0.02l	161.34 ± 0.27h	25.86 ± 0.21j	54.35 ± 0.25i
VA16 × LWS	130.21 ± 0.18h	17.62 ± 0.04k	164.53 ± 0.08h	27.33 ± 0.20g	61.24 ± 0.27f
VA16 × MWS	152.69 ± 0.14f	26.57 ± 0.04e	175.28 ± 0.24fh	33.56 ± 0.15c	73.39 ± 0.27d
VA16 × SWS	180.58 ± 0.12c	36.33 ± 0.06b	200.20 ± 0.34d	37.48 ± 0.18a	81.78 ± 0.24b
Variety					
VA6	82.23 ± 0.09d	13.88 ± 0.08d	190.69 ± 0.43d	14.33 ± 0.23d	31.74 ± 0.19d
VA11	122.54 ± 0.13c	28.31 ± 0.05b	182.10 ± 0.22b	29.39 ± 0.22b	66.48 ± 0.25b
VA14	179.03 ± 0.18a	28.72 ± 0.06a	311.54 ± 0.36a	27.52 ± 0.25c	57.61 ± 0.23c
VA16	148.01 ± 0.17b	24.07 ± 0.04c	175.34 ± 0.38c	31.06 ± 0.24a	67.69 ± 0.31a
SWC					
Control	116.53 ± 0.23d	18.34 ± 0.02d	193.28 ± 0.28d	22.27 ± 0.23d	45.66 ± 0.21d
LWS	118.56 ± 0.25c	19.31 ± 0.01c	196.49 ± 0.32c	22.98 ± 0.20c	49.04 ± 0.28c
MWS	139.59 ± 0.22b	23.84 ± 0.03b	221.06 ± 0.33b	26.54 ± 0.26b	59.58 ± 0.23b
SWS	157.13 ± 0.19a	33.48 ± 0.05a	248.83 ± 0.29a	30.52 ± 0.25a	69.23 ± 0.34a
Significance					
Variety	**	**	**	**	**
SWC	**	**	**	**	**
Variety × SWC	**	**	**	**	**

SWC = Soil water content, LWS = Low water stress, MWS = Medium water stress, SWS = Severe water stress, ** Significant at 1% level, TPC = Total polyphenol content (GAE µg g^{-1} dw), TFC = Total flavonoid content (RE µg g^{-1} dw), TAC (DPPH) = Total antioxidant capacity (DPPH) (TEAC µg g^{-1} dw), TAC ($ABTS^+$) = Total antioxidant capacity ($ABTS^+$) (TEAC µg g^{-1} dw), (n = 6)
In a column, mean values with different letters are differed significantly by Duncan multiple Range Test

These findings were partly in agreement with findings of Hanson *et al.* [74], who observed an increasing trend in beta-carotene content in Choysum variety but found a decreasing trend in beta-carotene content in Kailaan variety and decreasing trend in ascorbic acid content in both varieties from wet to dry season trial. The reason might be due to the differences in varieties. Similarly, Siracusa *et al.* [76] in buckwheat, Gharibi *et al.* [77] in *Achillea* species observed increasing trend in polyphenol, flavonoid content and antioxidant activity with the reduction of soil water content. For the interaction of variety × soil water content, betacyanin, betaxanthin and betalain content ranged from 160.02 to 499.01, 295.97 to 501.10 and 462.45 to 1000.46 ng g^{-1} FW, respectively.

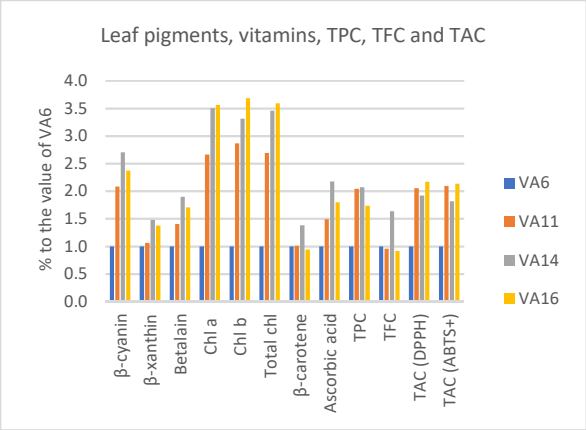

Fig. 5. Synthesis of leaf pigments, vitamins, TPC, TFC and TAC (% to the value of VA6) in selected *Amaranthus tricolor* genotypes, betacyanin(ng g^{-1}), betaxanthin (ng g^{-1}), betalain (ng g^{-1}), Chl a = chlorophyll a (mg g^{-1}), Chl b = chlorophyll b (mg g^{-1}), T chl = Total chlorophyll (mg g^{-1}), beta-carotene (mg g^{-1}), ascorbic acid (mg 100 g^{-1}), TPC = Total polyphenol content (GAE µg g^{-1} dw), TFC = Total flavonoid content (RE µg g^{-1} dw), TAC (DPPH) = Total antioxidant capacity (DPPH) (TEAC µg g^{-1} dw), TAC ($ABTS^{+}$) = Total antioxidant capacity ($ABTS^{+}$) (TEAC µg g^{-1} dw)

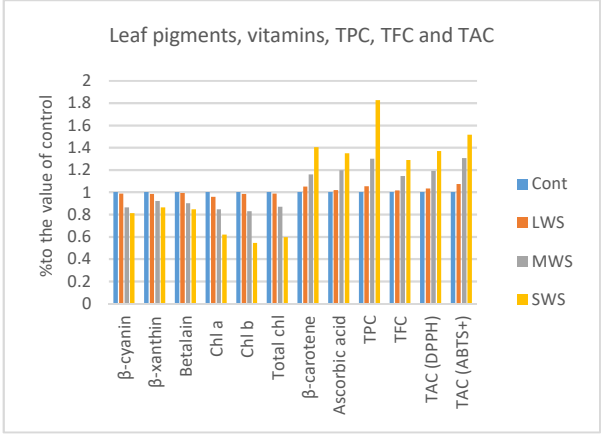

Fig. 6. Response of leaf pigments, vitamins, TPC, TFC and TAC (% to the value of control) under four soil water content: Control (100% FC), LWS (90% FC), MWS (60% FC), and SWS (30% FC), betacyanin(ng g^{-1}), betaxanthin (ng g^{-1}), betalain (ng g^{-1}), Chl a = chlorophyll a (mg g^{-1}), Chl b = chlorophyll b (mg g^{-1}), T chl = Total chlorophyll (mg g^{-1}), beta-carotene (mg g^{-1}), ascorbic acid (mg 100 g^{-1}), TPC = Total polyphenol content (GAE µg g^{-1} dw), TFC = Total flavonoid content (RE µg g^{-1} dw), TAC (DPPH) = Total antioxidant capacity (DPPH) (TEAC µg g^{-1} dw), TAC ($ABTS^{+}$) = Total antioxidant capacity ($ABTS^{+}$) (TEAC µg g^{-1} dw)

The highest betacyanin, betaxanthin and betalain content were observed in VA14 under control (499.01, 501.10 and 1000.46 ng g^{-1} FW) followed by VA14 under LWS, VA16 under control, VA16 under LWS and VA14 under MWS. VA6 under SWS (160.02, 295.97 and 462.45 ng g^{-1} FW) showed the lowest betacyanin, betaxanthin and betalain content. The highest chlorophyll a, chlorophyll b and total chlorophyll content were observed in VA14 under control (499.01, 261.38 and 768.47 µg g^{-1} FW) followed by VA14 under LWS, VA16 under control, VA16 under LWS and VA14 under MWS. In contrast, VA6 under SWS (160.02, 54.49 and 139.59 µg g^{-1} FW) showed the lowest chlorophyll a, chlorophyll b and total chlorophyll content. There was a significant decrease in betacyanin, betaxanthin, betalain, Chlorophyll a,

chlorophyll b and total chlorophyll content with the increment of soil water stress in all the varieties in this order: control > LWS > MWS > SWS.

Within the interaction of variety × soil water content, beta-carotene content ranged from 0.70 to 1.41 mg g^{-1} FW. The highest beta-carotene content was observed in VA14 under SWS (1.41 mg g^{-1} FW) followed by VA11 under SWS, VA4 under MWS and VA14 under LWS. The lowest beta-carotene content was observed in VA11 under control (0.70 mg g^{-1} FW). The highest ascorbic acid content was observed in VA14 under SWS (210.67 mg 100 g^{-1} FW) followed by VA14 under MWS, VA16 under SWS and VA14 under LWS. In contrast, VA6 under control (72.45 mg 100 g^{-1} FW) showed the lowest ascorbic acid content. Total polyphenol content ranged from 9.34 to 38.41 GAE µg g^{-1} dw. The highest total polyphenol content was observed in VA11 under SWS (38.41 GAE µg g^{-1} dw) followed by VA16 under SWS, VA14 under SWS and VA14 under MWS. In contrast, VA6 under control (9.34 GAE µg g^{-1} dw) showed the lowest total polyphenol content. Total flavonoid content ranged from 154.89 to 346.32 RE µg g^{-1} dw. The highest total flavonoid content was observed in VA14 under SWS (346.32 RE µg g^{-1} dw) and VA14 under MWS (335.86 RE µg g^{-1} dw) followed by VA14 under control, VA14 under LWS, VA11 under SWS and VA11 under MWS. In contrast, VA11 under control (154.89 RE µg g^{-1} dw) showed the lowest total flavonoid content. Total antioxidant capacity was ranged from 12.05 to 37.48 TEAC µg g^{-1} dw (DPPH) and 26.69 to 83. 34 TEAC µg g^{-1} dw ($ABTS^{+}$). The highest TAC (DPPH) was observed in VA16 under SWS (37.48 TEAC µg g^{-1} dw) followed by VA11 under SWS, VA16 under MWS and VA14 under SWS while, the highest TAC ($ABTS^{+}$) was observed in VA11 under SWS (83.34 TEAC µg g^{-1} dw) followed by VA16 under SWS, VA14 under SWS and VA16 under MWS. In contrast, VA6 under LWS (12.05 TEAC µg g^{-1} dw) showed the lowest TAC (DPPH) and VA6 under control (26.69 TEAC µg g^{-1} dw) showed the lowest TAC ($ABTS^{+}$). beta-carotene, ascorbic acid, TPC, TFC, TAC (DPPH) and TAC ($ABTS^{+}$) were significantly increased with the increment of soil water stress in all the varieties in the following order: control < LWS < MWS < SWS. Water stress interacted with varieties and elevated beta-carotene content in Choysum in dry season trial [74], in perennial herbaceous [75], TPC, TFC in buckwheat [76], TPC, TFC and antioxidant activity in *Achillea* species [77].

Correlation studies

Betacyanin had highly significant correlation with betaxanthin, betalain, chlorophyll a, chlorophyll b, total chlorophyll and ascorbic acid. This trait exhibited significant correlation with TAC (DPPH) and TAC ($ABTS^{+}$). Highly significant association of betaxanthin was observed with betalain, chlorophyll a, chlorophyll b, total chlorophyll and ascorbic acid.

betaxanthin was significantly interrelated with TFC. Betalain showed highly significant interrelationships with chlorophyll a, chlorophyll b, total chlorophyll and ascorbic acid. Similarly, this trait had significant correlation with TFC, TAC (DPPH) and TAC ($ABTS^+$). Chlorophyll a showed highly significant interrelationship with chlorophyll b, total chlorophyll and significant correlation with ascorbic acid TAC (DPPH) and TAC ($ABTS^+$). Highly significant interrelationship was noticed between chlorophyll b and total chlorophyll while, significant association was found between chlorophyll b and ascorbic acid and chlorophyll b and TAC (DPPH). Total chlorophyll was significantly associated with ascorbic acid, TAC (DPPH) and TAC ($ABTS^+$). beta-carotene displayed highly significant interrelationship with ascorbic acid, TPC and TFC while, this trait had significant associations with TAC (DPPH) and TAC ($ABTS^+$). Ascorbic acid demonstrated highly significant interrelationship with TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$). Highly significant associations were detected in TPC versus TAC (DPPH), TPC versus TAC ($ABTS^+$) and significant association between TPC and TFC. Significant association was observed between TFC and TAC (DPPH and $ABTS^+$). TAC (DPPH) was strongly and significantly associated with TAC ($ABTS^+$). Correlation study revealed that betacyanin, betaxanthin, betalain, chlorophyll a, chlorophyll b and total chlorophyll were strongly associated with each other along with TAC (DPPH and $ABTS^+$) indicating antioxidant activity of these traits. However, these traits had no association with β carotene, TPC and TFC. beta-carotene, ascorbic acid, TPC, TFC, TAC (DPPH) predominately interrelated with each other along with TAC ($ABTS^+$) revealed the antioxidant activity of these traits. Gharibi *et al*. [77] observed positive association among TPC, TFC and antioxidant activity in *Achillea* species.

Table 6. Correlation co-efficient for antioxidant leaf pigments, vitamin, TPC, TFC and TAC in four selected vegetable amaranth genotypes

	betaxanthin (ng g^{-1})	Betalain (ng g-1)	Chl a (µg g-1)	Chl b (µg g^{-1})	T chl (µg g^{-1})	β-carotene (mg g^{-1})	Ascorbic acid (mg 100 g^{-1})	TPC (GAE µg g^{-1} dw	TFC (RE µg g^{-1} dw)	TAC (DPPH) (TEAC µg g^{-1} dw)	TAC ($ABTS^+$) (TEAC µg g^{-1} dw)
betacyanin	0.86**	0.98**	0.91**	0.89**	0.92**	0.21	0.68**	0.38	0.39	0.56*	0.49*
betaxanthin		0.94**	0.89**	0.89**	0.90**	0.23	0.64**	0.11	0.45*	0.31	0.23
Betalain			0.90**	0.88**	0.91**	0.19	0.69**	0.29	0.43*	0.48*	0.51*
Chl a				0.96**	o.98**	-0.06	0.55*	0.25	0.16	0.52*	0.45*
Chl b					0.98**	-0.13	0.44*	0.14	0.10	0.43*	0.42
T Chl						-0.09	0.52*	0.21	0.14	0.49*	0.44*
beta-carotene							0.69**	0.61**	0.90**	0.43*	0.48*
Ascorbic acid								0.78**	0.75**	0.78**	0.74**
TPC									0.51*	0.86**	0.87**
TFC										0.56*	0.53*
TAC (DPPH)											0.98**

Chl a = Chlorophyll a, Chl b = Chlorophyll b, T chl = Total chlorophyll, TPC = Total polyphenol content (GAE µg g^{-1} dw), TFC = Total flavonoid content (RE µg g^{-1} dw), TAC (DPPH) = Total antioxidant capacity (DPPH) (TEAC µg g^{-1} dw), TAC ($ABTS^+$) = Total antioxidant capacity ($ABTS^+$) (TEAC µg g^{-1} dw), *significant at 5% level, ** significant at 1% level, (n = 6)

In this work, 4 cultivars of vegetable amaranth were selected from our germplasm collection and subjected to 4 irrigation regimes; significant changes in proximate composition, minerals, antioxidant leaf pigments, vitamins, TPC, TFC and antioxidant activity were observed. Based on the results reported (increase in most of the proximate compositions, mineral compositions, beta-carotene, ascorbic acid, TPC, TFC and antioxidant activity with the deficit of soil water content), this crop could be a promising alternative for farmers, especially in semi-arid and arid areas where water supply is scarce, as well as in dry seasons throughout the world.

Abstract

Four selected vegetable amaranths were grown under four soil water content to evaluate their response in nutrients, minerals, antioxidant leaf pigments, vitamins, polyphenol, flavonoid and total antioxidant activity (TAC). Vegetable amaranth was significantly affected by variety, soil water content and variety × soil water content interactions for all the traits studied. Increase in water stress, resulted in significant changes in proximate compositions, minerals (macro and micro), leaf pigments, vitamin, total polyphenol content (TPC), and total flavonoid content (TFC) of vegetable amaranth. Accessions VA14 and VA16 performed better for all the traits studied. Correlation study revealed a strong antioxidant scavenging activity of leaf pigments, ascorbic acid, TPC and TFC. Vegetable amaranth can tolerate soil water stress without compromising the high quality of the final product in terms of nutrients and antioxidant profiles. For this, it could be a promising alternative crop in semi-arid and dry areas and also during dry seasons.

3.1.2 Drought stress enhances nutritional and bioactive compounds, phenolic acids and antioxidant capacity of *Amaranthus* leafy vegetable

Purpose of the study

Both researchers and consumers have much interests to natural antioxidants of vegetables. These natural compounds protect many diseases, such as cancer, arthritis, emphysema, retinopathy, neuro-degenerative and cardiovascular diseases, atherosclerosis, and cataracts [3, 8, 35, 48]. *Amaranthus tricolor* is an inexpensive and excellent source of lots of natural antioxidants like nutritional and bioactive compounds, phenolics, flavonoids and detoxify reactive oxygen species (ROS) in human body [6, 35].

The intensity of damage caused by reactive oxygen species (ROS) mainly depends on its balance between production and elimination by the antioxidant scavenging system [64]. Moreover, drought stress favors rapid damage and leakage of plant cell membrane [64]. Environmental stresses cause oxidative damage in the plant. Stressed-plants have also protection systems to overcome the oxidative damage by synthesis of secondary metabolites like phenolics, flavonoids [65, 66]. These compounds can detoxify ROS in plants, and also have the capacity to cure many human diseases caused by oxidative damage and aging [67]. Amaranths can tolerate drought efficiently [68, 69]. *A. tricolor* is a well-acclimated leafy vegetable against biotic and abiotic stresses [70] and had multipurpose usages. Many processes, such as environmental, biological, ecological, physiological, biochemical and evolutionary process are involved in the quantitative and qualitative improvement of natural antioxidants of this species of which, drought stress can rapidly boost up the contents [72].

There is limited information on leafy vegetables regarding the effect of secondary metabolites to drought stress, such as nutritional and bioactive compounds, phenolics, flavonoids and antioxidants. Drought stress enhanced secondary metabolites, such as beta-carotene composition in Choysum varieties [74] and in perennial herbaceous [75], vitamin C in tomato [73], total polyphenol and total flavonoid content in buckwheat [79], total antioxidant activity, total polyphenol and total flavonoid content in *Achillea* species [77]. On the other hand, drought stress declined buckwheat's protein composition [79], beta-carotene composition of Kailaan variety [74] and vitamin C, Zn, Ca and Fe content of both varieties [74]. There is no literature regarding drought stress effects on nutritional and bioactive compounds, phenolics, flavonoids and antioxidant activity in *A. tricolor*. Our earlier studies [143, 149-151, 160-162, 173], we selected some antioxidants enriched and high yielding genotypes. Consequently, the study was aimed to evaluate the drought stress effects on selected

genotype for nutritional and bioactive compounds, phenolics, flavonoids and antioxidant activity.

Materials and methods

Experimental site, Plant materials and experimental conditions

Earlier, we collected 102 genotypes in different eco-geographical zones of the country. From this collection, an antioxidant enriched high yield potential genotype (Accession VA3) was selected based on our previous studies [143, 149-151, 160-162, 173]. This genotype was grown in pots under rain shelter open field of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. Pots were irrigated at 100% field capacity up to 5 days after planting (DAP) for dynamic growth and proper establishment of seedlings. After establishment period, *A. tricolor* plants were subjected to the different irrigation treatments as FC (100% field capacity, control), mild stress (90% FC), moderate stress (60% FC), and severe stress (30% FC). Throughout cultivation period, moisture levels in the soil were controlled by daily weighting following the standard procedure. Pots were weighed twice a day at 12 h intervals. To achieve the target field capacity of each water condition, the amount of water equaling that lost through transpiration and soil evaporation, percolation and leaching were added. Imposition of water stress was continued up to 30 DAP. At 30 DAP the leaves of *A. tricolor* were harvested from each experimental unit. All the parameters were measured in three replicates.

Chemicals

Solvent: methanol and acetone. Reagents: Standard compounds of pure phenolic acids, HPLC grade acetonitrile and acetic acid, vitamin C, gallic acid, rutin, methanol, DPPH (2,2-diphenyl-1-picryl-hydrazyl), $ABTS^{+}$(2,2-azinobis-3-ethyl-enzothiazoline-6-sulphonicacid), trolox (6-hydroxy-2,5,7,8-tetra-methyl-chroman-2-carboxylicacid), aluminum chloride hexa-hydrate, sodium carbonate, potassium acetate, Folin-Ciocalteu reagent, H_2SO_4, NaOH, HNO_3, $HClO_4$, lanthamum, Caesium chloride, dithiothreitol (DTT) and potassium persulfate. The pure and analytical grade solvents and reagents from Kanto Chemical Co. Inc. (Tokyo, Japan) and Merck (Germany) were used in this experiment.

Estimation of proximate composition, mineral content

Proximate composition and mineral content were measured following the procedure described in the previous chapter

Estimation of chlorophyll, total carotenoids, betacyanin and betaxanthin content

Chlorophyll, total carotenoids, betacyanin and betaxanthin content were measured following the procedure described in the previous chapter

Extraction of samples for TPC, TFC and TAC

Samples were extracted following the procedure described in the previous chapter

Estimation of beta-carotene, TPC, TFC and TAC

Beta-carotene, TPC, TFC and TAC were measured following the procedure described in the previous chapter

Extraction of samples for HPLC and LC-MS analysis

One gram of fresh-frozen leaves was homogenized with 10 ml of 80% methanol containing 1% acetic acid. The homogenized mixture was filtered through a 0.45 µm filter using a MILLEX®-HV syringe filter (Millipore Corporation, Bedford, MA, USA) and centrifuged at 10,000g for 15 min. The final filtrate was used to analyze phenolic acids and flavonoids.

HPLC analysis of phenolic acids and flavonoids

The amounts of phenolic acids and flavonoids in *A. tricolor* leaf sample were measured using HPLC with the method described by Khanam *et al*. [174] The HPLC system (Shimadzu SCL10Avp, Kyoto, Japan) was equipped with LC-10Avp binary pumps, a degasser (DGU-14A) and a variable Shimadzu SPD-10Avp UV–vis detector. Phenolic acids and flavonoids were separated by a CTO-10AC (STR ODS-II, 150 × 4.6 mm I.D., Shinwa Chemical Industries, Ltd., Kyoto, Japan) column. The binary mobile phase consisted of 6% (v/v) acetic acid in water (solvent A) and acetonitrile (solvent B) was pumped at a flow rate of 1 ml/min for a total run time of 70 min. The system was run with a gradient program: 0–15% B for 45 min, 15–30% B for 15 min, 30–50% B for 5 min and 50–100% B for 5 min. The injection volume was 10 µl while the column temperature was maintained at 35 °C. The detector was set at 254, 280 and 360 nm for simultaneous monitoring of hydroxybenzoic acids, hydroxycinnamic acids and flavonoids. The compound was identified by comparing their retention time and UV–vis spectra with those of standards. The phenolic acids and flavonoids were also qualitatively confirmed using mass spectrometry. The sum of concentrations of all phenolic acids and flavonoids, quantified by HPLC, was denoted as the total phenolic index (TPI). From the HPLC data, TPI was obtained according to the method described by Khanam *et al*. [174]. All samples were prepared and analyzed in duplicate. The results were expressed as µg g^{-1} fresh weight (FW).

The Mass spectrometry analyses were performed in the negative ion mode using a JEOL AccuTOF (JMS-T100LP, JEOL Ltd., Tokyo, Japan) mass spectrometer fitted with an Agilent 1100 Series HPLC system and a UV–vis detector coupled on-line with an ElectroSpray

Ionization (ESI) source. The column elutes were recorded in the range of m/z 0-1000. Needle voltage was kept at -2000 V. The chromatographic conditions were optimized to obtain chromatograms with good resolution of adjacent peaks, for which a slight modification was made in the method reported by Khanam *et al*. [174]. Extract constituents were identified by LC-MS-ESI analysis.

Statistical Analysis

Data were analyzed according to the procedure described in the previous chapter

Results and discussion

Influence of nutritional compositions to drought stress

Effects of nutritional compositions under different drought stress of A. tricolor are presented in Fig. 1. Control and low drought stress (LDS) condition exhibited the highest moisture content, while the moisture content was gradually decreased from moderate drought stress (MDS) to severe drought stress (SDS). Moisture content was drastically reduced with the increase of drought stress in the order: (control > LDS > MDS > SDS). SDS condition had the highest protein, ash, and dietary fiber content, while the lowest protein, ash, and dietary fiber content were observed under the control condition.

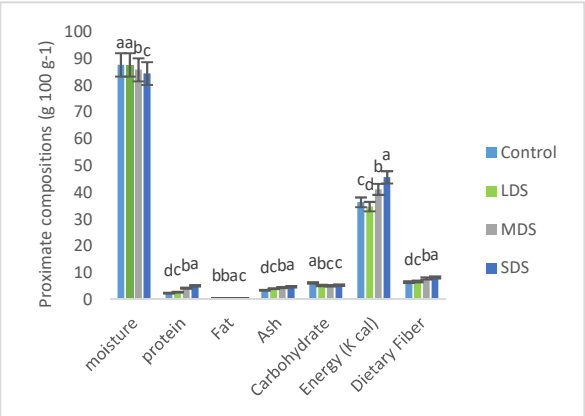

Fig. 1. Changes of proximate compositions (g 100 g^{-1}) at four drought levels: Control (100% FC), LDS (90% FC), MDS (60% FC), and SDS (30% FC) in a selected *A. tricolor* genotype; (n = 3), letters mentioned in the bars are significantly varied by DMRT (P < 0.01)

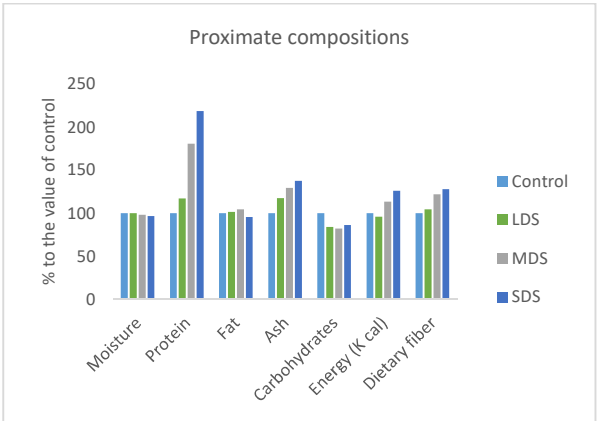

Fig. 2. Effect of proximate composition (g 100 g^{-1}) (% to the value of control) at four drought levels: Control (100% FC), LDS (90% FC), MDS (60% FC), and SDS (30% FC) in a selected *A. tricolor* genotype

Protein, ash and dietary fiber content were remarkably increased with an increase in the severity of drought stress in the following order: control < LDS < MDS < SDS. In LDS, MDS and SDS, protein, ash and dietary fiber content were augmented by (17%, 17% and 4%); (80%, 29% and 21%) and (118%, 38% and 28%); respectively over control condition (Fig. 2). MDS condition had the highest fat content, and the lowest fat content was recorded at SDS condition,

while the intermediate fat content was noticed under control and LDS conditions. Control condition had the highest carbohydrates content and it was gradually decreased in the order: control > LDS > MDS = SDS, which was statistically similar to MDS and SDS, respectively. Carbohydrate content was sharply declined with the severity of drought stress. The energy ranged from 34.67 to 45.61 g 100 g^{-1} with the highest energy was recorded in SDS and the lowest in LDS condition.

As leafy vegetables, *A. tricolor* leaves exhibited high moisture content. Nevertheless, it demonstrated a noble source of protein, dietary fiber, carbohydrates and ash. Moisture content was significantly reduced with the increment of drought stress in the following order: (control > LDS > MDS > SDS). As lower moisture contents of leaves ensured higher dry matter, the drought-stressed plant could be a promising source of dry matter compared to control condition. In LDS, MDS and SDS, protein, ash and dietary fiber content were augmented by (17%, 17% and 4%); (80%, 29% and 21%) and (118%, 38% and 28%); respectively over control condition. However, Siracusa *et al*. [76] observed a decrease in protein content at drought stress to fully irrigated in buckwheat. The genotypic variances between two crops might be contributed for the different results. *A. tricolor* is the sources of protein for vegetarian and poor people of the third world countries. Dietary fiber has a significant role in palatability, digestibility and remedy of constipation [151]. MDS condition had the highest fat content, and the lowest fat content was observed under SDS condition. Fats are sources of omega-3 and omega-6 fatty acids. It helps in the digestion, absorption, and transport of fat-soluble vitamins A, D, E, and K. Sun *et al*. [175] observed similar results in sweet potato leaves where they mentioned that fat involved in the insulation of body organs and in the maintenance of body temperature and cell function. Control had the highest carbohydrates content and it was gradually decreased in the order: control > LDS > MDS = SDS, which was statistically similar to MDS and SDS, respectively. Carbohydrate content sharply declined with the severity of drought stress. As a leafy vegetable, the low carbohydrate content of amaranth leaves has no a substantial effect in the daily diet of the human body. As regards energy balance, SDS exhibited remarkably higher calories compared to MDS, LDS and control conditions, while these variations have no remarkable impact on the daily diet of the human body, since very little amounts were consumed in the daily diet. Drought stress increased the protein, ash, energy, fat and dietary fiber content and reduced carbohydrate and moisture content of *Amaranthus* leaves. For this, *Amaranthus* leafy vegetable might be produced in a semi-arid and dry area in the world could be contributed as a noble source of dietary fiber and vegetarian protein in the human diet.

Drought stress effects on mineral content

Results of minerals (macro and microelements) contents are presented in Fig. 3 and 4. The mineral contents of *A. tricolor* were progressively influenced by drought stress.

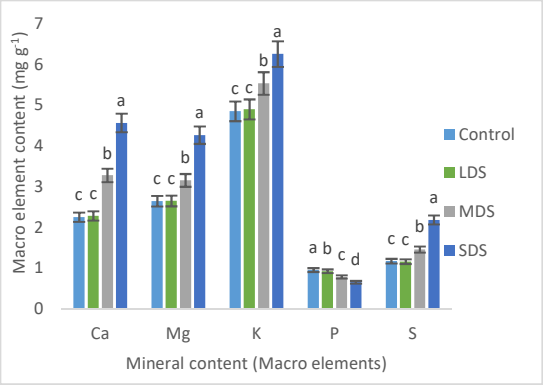

Fig. 3. Response of mineral content (Macro elements mg g^{-1}) at four drought levels: Control (100% FC), LDS (90% FC), MDS (60% FC), and SDS (30% FC) in a selected *A. tricolor* genotype; (n = 3), letters mentioned in the bars are significantly varied by DMRT (P < 0.01)

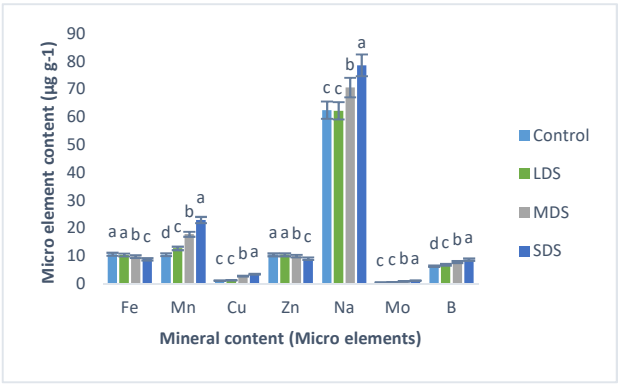

Fig. 4. Impact of mineral content (Micro elements µg g^{-1}) at four drought levels: Control (100% FC), LDS (90% FC), MDS (60% FC), and SDS (30% FC) in a selected *A. tricolor* genotype; (n = 3), letters mentioned in the bars are significantly varied by DMRT (P < 0.01)

Ca, K, Na, Mg, S, Cu and Mo content were statistically similar under control and LDS conditions, whereas Ca, Mg, K, S, Cu, Na and Mo content were sharply and remarkably augmented with the severity of drought stress from MDS and SDS conditions showing the order: control = LDS < MDS < SDS. In MDS and SDS, Ca, K, S, Mg, Na, Cu and Mo content were augmented by (46%, 19%, 14%, 25%, 148%, 13% and 119%) and (103%, 61%, 29%, 86%, 215%, 26% and 200%), respectively compared to control and LDS conditions (Fig. 5). B and Mn content were statistically increased with the increase of drought stress in the order: control <LDS < MDS < SDS. In LDS, MDS and SDS, the rate of increase of Mn and B were (22%, 8%), (71%, 23%) and (121%, 37%), respectively, over the control condition (Fig. 5). Further, it was noted that, increasing drought stress lead to a significant decrease in P content in the following order: control > LDS > MDS > SDS. In LDS, MDS and SDS, reduction of P was 3%, 18% and 32%, respectively, over control condition (Fig. 5). Statistically, there were no significant differences in Fe and Zn content under control and LDS conditions, whereas Fe and Zn content were significantly and drastically declined with the severity of drought stress from MDS and SDS conditions showing the order: control = LDS > MDS > SDS. In MDS and SDS, Fe and Zn content were reduced by (8%, 5%) and (17%, 13%), respectively compared to control and LDS conditions (Fig. 5). The highest Ca, Mg, K, S, Mn, Cu, Na, Mo and B content were observed in SDS, while the lowest Ca, Mg, K, S, Mn, Cu, Na, Mo and B content were

found in control or LDS condition. Conversely, the highest P, Fe and Zn content were recorded in control or LDS condition and the lowest P, Fe and Zn content were found in SDS.

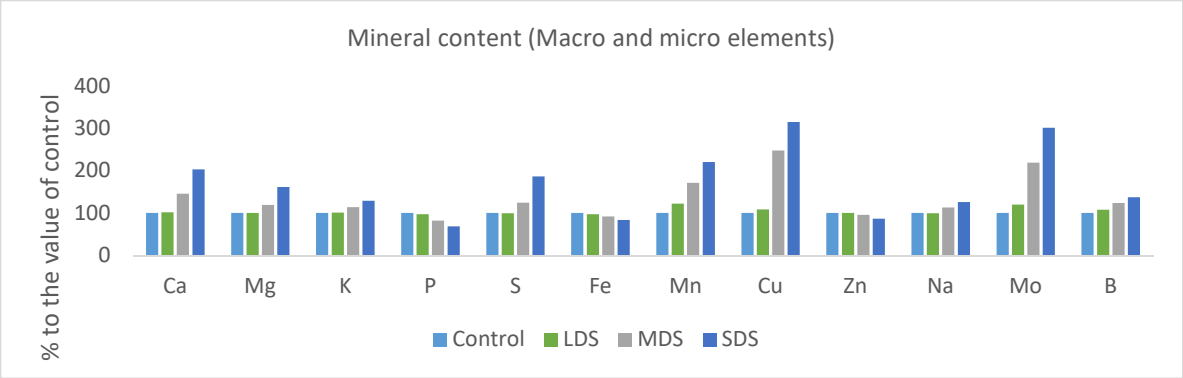

Fig. 5. Assessment of mineral contents (Macro and microelements, mg g^{-1} and µg g^{-1}, respectively) (% to the value of control) at four drought levels: Control (100% FC), LDS (90% FC), MDS (60% FC), and SDS (30% FC) in a selected *A. tricolor* genotype

Amaranth leaves are noble sources of minerals (macro and microelements). The mineral content of amaranth leaves was remarkably influenced by drought stress. Zinc and Fe content of *A. tricolor* are greater than that of the cassava leaves [176] and beach pea [177]. Similarly, Jimenez-Aguiar & Grusak [178] reported high Fe, Mn, Cu and Zn (fresh weight basis) in different *A. spp.* including *A. tricolor*. They also reported that Amaranths had higher Zn content than black nightshade, spinach and kale; more Fe and Cu content than kale. Ca, Mg, K, S, Cu, Na and Mo content were sharply and remarkably augmented with the severity of drought stress from MDS and SDS conditions showing the order: control = LDS < MDS < SDS. On the other hand, Hanson *et al.* [74] reported a decline in Ca content both in Choysum and Kailaan varieties from dry season to wet season trial. SDS exhibited the highest Ca, K, S, Mg, Mn, Na, Cu, Mo and B content, while control or LDS condition had the lowest Ca, S, K, Mg, Cu, Mn, Mo, Na and B content. In contrast, control or LDS condition had the highest Zn, P, and Fe content and SDS exerted the lowest P, Zn and Fe content. Likewise, Hanson *et al*. [74] recorded a decline in Fe content of Choysum variety, whereas they reported a sharp increment in Kailaan variety from dry season to wet season trial. Moreover, Hanson *et al*. [74] recorded a remarkable increment in Zn content both in Kailaan and Choysum variety from dry season to wet season trial. Except P, Fe and Zn, all the mineral content were progressively raised with the increment of drought stress, whereas, Zn, Fe and P were sharply declined with the increment of drought stress. Therefore, *A. tricolor* cultivated in a drought-stressed area specifically in semi-arid and drought-prone area could be contributed as a noble source of minerals content in the daily diet of the human body related to usual farming practices.

Drought stress effects on leaf pigments

Leaf pigments of vegetable amaranth were significantly affected by drought stress (Fig.6). Except total carotenoids, all the leaf pigments (Betacyanin, betaxanthin, betalain, chlorophyll *a*, chlorophyll *b* and chlorophyll *ab* content) were significantly and gradually reduced with the increase of the severity of drought stress (control > LDS > MDS > SDS). In LDS, MDS and SDS, betacyanin, betaxanthin, betalain, chlorophyll *a*, chlorophyll *b* and chlorophyll *ab* content were declined by (0.5%, 2%, 1%, 3%, 2% and 3%); (5%, 5%, 5%, 7%, 9% and 8%) and (8%, 9%, 9%, 12%, 12% and 12%); respectively, over control condition (Fig. 7). Betacyanin, betaxanthin, betalain, chlorophyll *a*, chlorophyll *b* and chlorophyll *ab* content were the highest in control condition, whereas betacyanin, betaxanthin, betalain, chlorophyll *a*, chlorophyll *b* and chlorophyll *ab* content were the lowest in SDS.

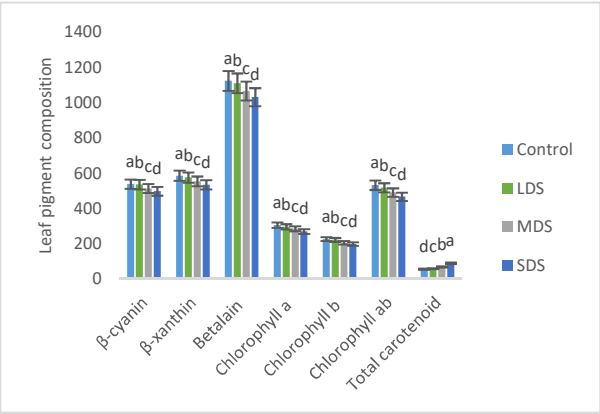

Fig. 6. Influence of Leaf pigments at four drought levels: Control (100% FC), LDS (90% FC), MDS (60% FC), and SDS (30% FC) in a selected *A. tricolor* genotype; Betacyanin (ng g^{-1} FW), Betaxanthin (ng g^{-1} FW), Betalain (ng g^{-1} FW), Chlorophyll a (µg g^{-1} FW), Chlorophyll b (µg g^{-1} FW), Chlorophyll ab (µg g^{-1} FW), Total carotenoids (mg 100 g^{-1} FW); (n = 3), letters mentioned in the bars are significantly varied by DMRT ($P < 0.01$)

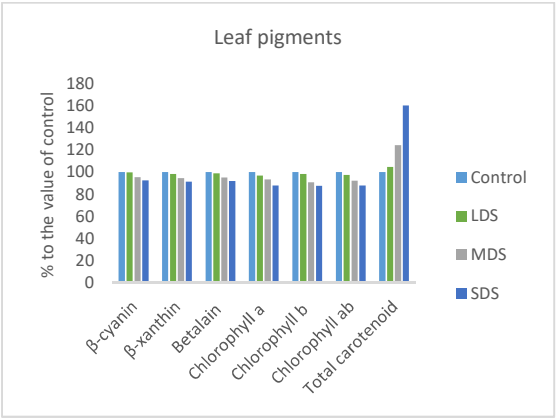

Fig. 7. Comparison of leaf pigments (% to the value of control) at four drought levels: Control (100% FC), LDS (90% FC), MDS (60% FC), and SDS (30% FC) in a selected *A. tricolor* genotype; Betacyanin (ng g^{-1} FW), Betaxanthin (ng g^{-1} FW), Betalain (ng g^{-1} FW), Chlorophyll a (µg g^{-1} FW), Chlorophyll b (µg g^{-1} FW), Chlorophyll ab (µg g^{-1} FW),Total carotenoids (mg 100 g^{-1} FW)

Total carotenoids were significantly and remarkably increased with the increasing the severity of drought stress (control < LDS < MDS < SDS). In LDS, MDS and SDS, total carotenoids were significantly and remarkably increased by 4%, 24% and 60%, respectively over the control condition (Fig. 7). Leaf pigments of *A. tricolor* were statistically influenced by drought stress. Except total carotenoids, all the leaf pigments (Betacyanin, betaxanthin, betalain, chlorophyll *a*, chlorophyll *b* and chlorophyll *ab* content) were significantly and gradually reduced with the increasing the severity of drought stress (control > LDS > MDS > SDS). Likewise, Hsu and Kao [172] reported a decline in chlorophyll content with the increment of drought severity. They also stated that drought stress influenced growth and

development of plant through osmotic stress, declining the water potential, reducing stomatal conductivity which limits CO_2 influx to leaves and unfavorable CO_2/O_2 ratio in chloroplasts, decreasing photosynthesis.

Influence of beta-carotene, vitamin C, TPC, TFC and TAC to drought stress

Beta-carotene, vitamin C content, TPC, TFC and TAC of *A. tricolor* were progressively influenced by drought stress Fig.8.

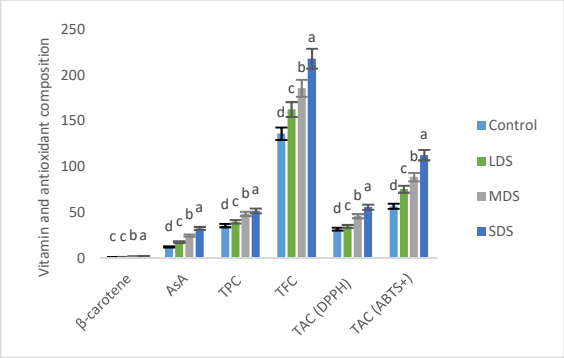

Fig. 8. Response of Beta-carotene, Vitamin C, TPC, TFC and TAC at four drought levels: Control (100% FC), LDS (90% FC), MDS (60% FC), and SDS (30% FC) in a selected *A. tricolor* genotype; AsA, Vitamin C (mg 100 g^{-1}); Beta-carotene (mg g^{-1}), TFC, Total flavonoid content (RE µg g^{-1} dw);TPC, Total polyphenol content (GAE µg g^{-1} dw); TAC (DPPH), Total antioxidant capacity (DPPH) (TEAC µg g^{-1} dw); TAC ($ABTS^+$), Total antioxidant capacity ($ABTS^+$) (TEAC µg g^{-1} dw); (n = 3), letters mentioned in the bars are significantly varied by DMRT ($P < 0.01$)

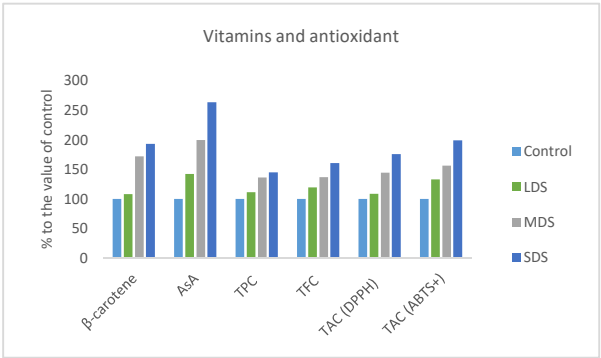

Fig. 9. Response of Vitamins, TFC,TPC and TAC, (% to the value of control) at four drought levels: Control (100% FC), LDS (90% FC), MDS (60% FC), and SDS (30% FC) in a selected *A. tricolor* genotype; AsA, Vitamin C (mg 100 g^{-1}); Beta-carotene (mg g^{-1}), TFC, Total flavonoid content (RE µg g^{-1} dw);TPC, Total polyphenol content (GAE µg g^{-1} dw); TAC ($ABTS^+$), Total antioxidant capacity ($ABTS^+$); (TEAC µg g^{-1} dw)TAC (DPPH), Total antioxidant capacity (DPPH) (TEAC µg g^{-1} dw)

In this investigation, beta-carotene, vitamin C content, total polyphenol content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC) (DPPH) and TAC ($ABTS^+$) were significantly increased with the increasing of the severity of drought stress in the order: control < LDS < MDS < SDS. In LDS, MDS and SDS, beta-carotene, vitamin C content, TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) were augmented by (8%, 42%, 11%, 19%, 9% and 33%); (72%, 100%, 36%, 37%, 45% and 56%) and (93%, 63%, 45%, 60%, 75% and 99%); respectively, compared to control condition (Fig. 9). SDS condition had the highest beta-carotene, vitamin C, TPC, TFC, TAC, (DPPH) and TAC ($ABTS^+$), while the control condition exhibited the lowest beta-carotene, vitamin C, TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$). Hanson *et al.* [74] reported an increase in beta-carotene content of Choysum variety. In contrast, they reported a declining trend in beta-carotene content of Kailaan variety and reduction in vitamin C content in both varieties from dry to wet season trial. The reason for reduction might be due to the genotypic variations in two different crops. Likewise, Gharibi *et al.* [77] in *Achillea* species and Siracusa *et al.* [76] in buckwheat, reported increment in antioxidant activity, polyphenol and flavonoid content with the severity of drought stress. The ameliorate

response of beta-carotene content with the severity of drought stress was also reported in Choysum varieties in dry season trial [74], and in perennial herbaceous [75]. Siracusa *et al.* [76] reported an increment of TPC, TFC in buckwheat with increasing the drought stress. Garibi *et al*. [77] also reported the enhancing response of TPC, TFC and antioxidant activity in *Achillea* species with the increment of drought stress.

Influence of drought stress on phenolics and flavonoids

Results of retention time, λmax, molecular ion, main fragment ions in MS^2 and tentative compound identification for phenolic compounds are presented in Table 1.

Table 1. Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, tentative identification of phenolic compounds and quantification ($\mu g g^{-1}$ FW) in *Amaranthus tricolor* leaves.

Phenolic compound	Rt (min)	λ_{max} (nm)	Molecular ion $[M - H]^-$ (m/z)	Identity	MS^2 (m/z)	Control (100% FC)	LDS (90%FC)	MDS (60% FC)	SDS (30% FC)
Hydroxybenzoicacid									
Gallic acid	9.1	254	169	3,4-5Trihydroxybenzoicacid	169.2	7.23 ± 0.03d	8.64 ± 0.04c	10.25 ± 0.05b	12.25 ± 0.06a
Vanilic acid	30.6	254	167	4-hydroxy-3-methoxybenzoicacid	167.2	9.75 ± 0.07d	10.12 ± 0.05c	12.83 ± 0.04b	15.48 ± 0.08a
Syringic acid	34.8	254	197	4-Hydroxy-3,5-dimethoxybenzoicacid	197.1	1.17 ± 0.02c	1.22 ± 0.01c	1.65 ± 0.02b	1.83 ±0.01a
p-hydroxybenzoic acid	31.5	254	137	4-hydroxybenzoic acid	137.2	2.64 ± 0.03d	2.84 ± 0.02c	3.26 ± 0.02b	4.07 ± 0.03a
Salicylic acid	48.2	254	137	2-Hydroxybenzoic acid	137.2	17.45 ±0.21d	18.96 ± 0.12c	25.68 ± 0.14b	28.96 ± 0.16a
Ellagic acid	52.5	254	301	(2,3,7,8-tetrahydroxy-chromeno [5,4,3-cde]chromene-5,10-dione	301.1	0.98 ± 0.01d	1.04 ± 0.02c	2.15 ± 0.02b	2.08 ± 0.03a
Total benzoic acids						39.22	42.81	55.81	64.66
Hydroxycinnamic acid									
Caffeic acid	32.0	280	179	3,4-Dihydroxy-trans-cinnamate	179.1	1.56 ± 0.02d	1.68 ± 0.01c	1.96 ± 0.02b	2.68 ± 0.03a
Chlorogenic acid	31.1	280	353	3-(3,4-Dihydroxycinnamoyl) quinic acid	353.2	9.86 ± 0.18d	10.26 ± 0.24c	12.54 ± 0.26b	13.86 ± 0.20a
p-coumaric acid	42.0	280	163	4-hydroxycinnamicacid	163.1	1.04 ± 0.02d	1.12 ± 0.01c	2.14 ± 0.02b	2.24 ± 0.02a
Ferulic acid	47.9	280	193	4-hydroxy-3-methoxycinnamicacid	193.2	1.02 ± 0.01c	1.08 ± 0.02c	1.55 ± 0.01b	2.15 ± 0.03a
m-coumaric acid	49.6	280	163	3-hydroxycinnamicacid	163.3	3.13 ± 0.03d	3.54 ± 0.02c	6.55 ±0.04b	7.96 ± 0.05a
Sinapic acid	49.0	280	223	4-Hydroxy-3,5-dimethoxycinnamicacid	223.2	0.26 ± 0.01d	0.34 ± 0.01c	0.38 ± 0.01b	0.42 ± 0.01a
Trans-cinnamic acid	67.3	280	147	3-Phenylacrylic acid	147.1	5.03 ± 0.02d	5.26 ± 0.01c	5.54 ± 0.01b	5.65 ±0.02a
Total cinnamic acids						21.89	23.27	31.66	34.96
Flavonoids									
Iso-quercetin	54.3	360	463	Quercetin-3-glucoside	463.3	3.55 ± 0.02c	3.58 ± 0.03c	6.46 ± 0.02b	7.82 ± 0.04a
Hyperoside	53.3	360	463	Quercetin-3-galactoside	463.5	1.18 ± 0.01c	1.22 ± 0.02c	1.58 ± 0.01b	2.05 ± 0.02a
Rutin	53.0	360	609	Quercetin-3-rutinoside	609.4	7.89 ± 0.06c	7.96 ± 0.05c	9.58 ± 0.06b	11.24 ± 0.04a
Total flavonoids						57.11	62.08	82.47	95.62
Total phenolic acids						16.59	18.76	21.62	25.12
Total phenolic index						73.70	78.84	104.09	120.74

Different letters in a row are differed significantly by Duncan Multiple Range Test ($P < 0.01$); ($n = 3$)

The values of phenolic acids and flavonoids components of *A. tricolor* genotype VA3 were separated though LC by comparing with masses of ion of standard flavonoids and phenolic acids and also by detecting the specific peaks of the corresponding components. A total of sixteen phenolic compounds were identified including six hydroxybenzoic acids, seven hydroxycinnamic acids and three flavonoids. *Trans*-cinnamic acid was newly identified phenolic acid in *A. tricolor*. However, an attempt was made for the first time to study the effect of drought stress in antioxidant enriched and high yield potential *A. tricolor* genotype VA3, in terms of sixteen phenolic acids and flavonoids. Within phenolic acids and flavonoids, hydroxybenzoic acids were identified as most abundant compounds in this genotype. Among hydroxybenzoic acids, salicylic acid was identified as one of the main phenolic acids followed by vanilic acid and gallic acid and *p*-hydroxybenzoic acid. Considering hydroxycinnamic acids, chlorogenic acid and *Trans*-cinnamic acid were the most abundant compound followed by *m*-

coumaric acid. A good amount of caffeic acid, *p*-coumaric acid and ferulic acid were also identified in this genotype. In this investigation, the flavonoids, rutin (quercetin-3-rutinoside) and isoquercetin (quercetin-3-glucoside) were the most abundant in this genotype.

The hydroxybenzoic acid (Syringic acid and); the hydroxycinnamic acid (Ferulic acid) and three flavonoids, iso-quercetin, hyperoside and rutin had no significant differences in their compositions under control and LDS conditions, nevertheless, the composition of these acids were significantly increased from MDS to SDS. In MDS and SDS, these phenolic acids and flavonoids compositions were increased by (41%, 53%, 82% 34% and 22%) and (56%, 111%, 121% 74% and 43%); respectively compared to control or LDS condition (Fig.10 and 11). Five hydroxybenzoic acids (Gallic acid, vanilic acid, *p*-hydroxybenzoic acid, salicylic acid and ellagic acid) and six hydroxycinnamic acid (Caffeic acid, chlorogenic acid, *trans*-cinnamic acid, *p*-coumaric acid, *m*-coumaric acid and sinapic acid) were remarkably increased with the increment of the severity of drought stress in the order: Control< LDS < MDS < SDS. In LDS, MDS and SDS, these phenolic acids and flavonoids concentrations were increased by (19%, 4%, 7%, 9% 6%, 8%, 4%, 8%, 13%, 31% and 22%); (42%, 32%, 23%, 19%, 42%, 26%, 27%, 105%, 109%, 47% and 50%) and (69%, 59%, 54%, 66% 111%, 65%, 72%, 41%, 115%, 45%, 154%, 65% and 60%); respectively (Fig.10 and 11). A total of sixteen phenolic compounds were identified including six hydroxybenzoic acids, seven hydroxycinnamic acids and three flavonoids. *Trans*-cinnamic acid was newly identified phenolic acid in *A. tricolor*.

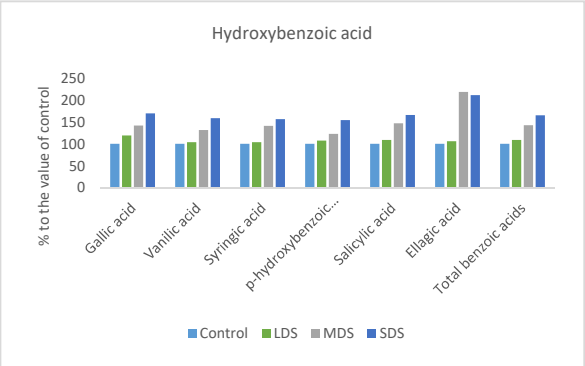

Fig. 10. Changes of hydroxybenzoic acid compositions (µg g^{-1} FW) (% to the value of control) at four drought levels: Control (100% FC), LDS (90% FC), MDS (60% FC), and SDS (30% FC) in a selected *A. tricolor* genotype

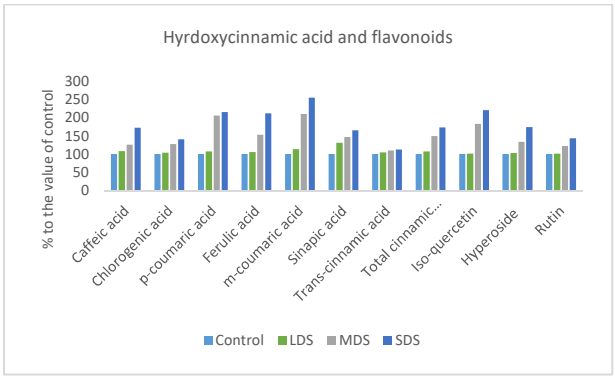

Fig. 11. Changes of hydroxycinnamic acid and flavonoid compositions (µg g^{-1} FW) (% to the value of control) at four drought levels: Control (100% FC), LDS (90% FC), MDS (60% FC), and SDS (30% FC) in a selected *A. tricolor* genotype

Khanam & Oba [179] in red and green amaranths and Khanam *et al*. [174] in eight different leafy vegetables including amaranths described rest fifteen phenolic acids and flavonoids with normal cultivation practices. However, an attempt was made for the first time

to study the effect of drought stress in antioxidant enriched and high yield potential *A. tricolor* genotype VA3, in terms of sixteen phenolic acids and flavonoids. Gallic acid, vanilic acid and *p*-hydroxybenzoic acid content of the genotype VA3 under control condition were higher than *A. tricolor* genotypes that reported by Khanam *et al.* [174]. Considering hydroxycinnamic acids, chlorogenic acid and *trans*-cinnamic acid were the most abundant compound followed by *m*-coumaric acid. A good amount of caffeic acid, *p*-coumaric acid and ferulic acid were also identified in this genotype. Under control condition, chlorogenic acid, caffeic acid and *m*-coumaric acid of this genotype was higher than *A. tricolor* genotypes reported by Khanam et al. [174]. The hydroxycinnamic acids synthesized from phenylalanine are the most extensively disseminated phenolic acids in plant tissues [180]. In plants, flavonoids occasionally occur as a glycone, although the most common forms are glycoside derivatives. These compounds account for 60% of total dietary phenolic compounds [181]. Flavonols are the most prevalent flavonoids in the plant kingdom and glycosides of quercetin are the most predominant naturally occurring flavonols [181]. In this investigation, the flavonoids, rutin (quercetin-3-rutinoside) and isoquercetin (quercetin-3-glucoside) were the most abundant in this genotype. The genotype VA3 exhibited higher rutin (quercetin-3-rutinoside) content under control condition in comparison to *A. tricolor* genotypes that reported by Khanam *et al*. [174]. All the phenolic acids and flavonoids had the lowest concentrations under control condition, whereas these acids exhibited the highest concentrations under SDS condition. Hence, *A. tricolor* cultivated in a drought-stressed area specifically in the semi-arid and drought-prone area could be contributed as the noble source of minerals and bioactive compounds, phenolics and flavonoid content and antioxidant activity in the daily diet of the human body related to usual farming practices.

In this study, antioxidant enriched and high yield potential *A. tricolor* genotype VA3 was selected from our germplasm collection and evaluated for nutritional and bioactive compounds, phenolic acids, flavonoids and antioxidant capacity under 4 irrigation regimes. *Trans*-cinnamic acid was newly identified phenolic acid in *A. tricolor*. Drought stress resulted in significant increment in protein, ash, energy, dietary fiber, K, S, Ca, Mn, Mg, Na, Cu, Mo and B content, total carotenoids, beta-carotene, vitamin C, TAC (DPPH), TFC, TPC and TAC ($ABTS^{+}$), sixteen phenolic acids and flavonoids. All the nutritional and bioactive compounds, phenolics, flavonoids and antioxidant capacity of *A. tricolor* leaves was very high under MDS and SDS condition, in comparison to control condition, that could be contributed as valuable food sources for human diets and health benefit. Nutritional and bioactive compounds, phenolics, flavonoids might be played a vital role in scavenging ROS and would be beneficial for human nutrition by serving as good antioxidants and antiaging sources in human health

benefit. Moreover, *A. tricolor* cultivated under drought stress could be contributed as a quality product of nutritional and bioactive compounds, phenolics, flavonoids and antioxidants. Based on the results reported farmers of semi-arid and dry areas of the world could be able to grow amaranth as an alternative crop.

Abstract

Bioactive compounds, vitamins, phenolic acids, flavonoids of *A. tricolor* are the sources of natural antioxidant that had a great importance for the food industry as these detoxify ROS in the human body. These natural antioxidants protect human from many diseases such as cancer, arthritis, emphysema, retinopathy, neuro-degenerative cardiovascular diseases, atherosclerosis and cataracts. Moreover, previous literature has shown that drought stress elevated bioactive compounds, vitamins, phenolics, flavonoids and antioxidant activity in many leafy vegetables. Hence, we study nutritional and bioactive compounds, phenolic acids, flavonoids and antioxidant capacity of amaranth under drought stress for evaluation of the significant contribution of these compounds in the human diet. The genotype VA3 was assessed at four drought stress levels that significantly affected nutritional and bioactive compounds, phenolic acids, flavonoids and antioxidant capacity. Protein, ash, energy, dietary fiber, Ca, K, Cu, S, Mg, Mn, Mo, Na, B content, total carotenoids, TFC, vitamin C, TPC, TAC (DPPH), beta-carotene, TAC ($ABTS^{+}$), sixteen phenolic acids and flavonoids were remarkably increased with the severity of drought stress. At moderate and severe drought stress conditions, the increments of all these components were more preponderant. *Trans*-cinnamic acid was newly identified phenolic acid in *A. tricolor*. Salicylic acid, vanilic acid, gallic acid, chlorogenic acid, *trans*-cinnamic acid, rutin, isoquercetin, *m*-coumaric acid and *p*-hydroxybenzoic acid were the most abundant phenolic compounds in this genotype. In *A. tricolor,* drought stress enhanced the quantitative and qualitative improvement of nutritional and bioactive compounds, phenolic acids, flavonoids and antioxidants. Hence, farmers of semi-arid and dry areas of the world could be able to grow amaranth as a substitute crop.

3.2 Biochemistry and Physiological Aspect on Drought Stress of Vegetable Amaranth

Drought stress leads to the accumulation of reactive oxygen species (ROS), which might initiate destructive oxidative processes such as lipid peroxidation, chlorophyll and betalain bleaching and protein oxidation. Plants have evolved both enzymatic and non-enzymatic defense systems for scavenging and detoxifying ROS, resulting in antioxidant defense capacity [78]. Nonenzymatic antioxidants [metabolites such as ascorbate (AsA), carotenoids, glutathione (GSH), phenolics, flavonoids and proline] and antioxidant enzymes [such as guaiacol peroxidases (GPOX), catalase (CAT), superoxide dismutase (SOD) and AsA-GSH cycle enzymes like glutathione reductase (GR) ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR)], work together for detoxification of ROS [88, 89].

3.2.1 Drought stress effects on growth, ROS markers, compatible solutes, phenolics, flavonoids, and antioxidant activity in *Amaranthus tricolor*

Purpose of the study

Amaranthus tricolor L. is one of the most important and popular leafy vegetables in Bangladesh including Southeast Asia, Africa and South America often cultivated in arid and semiarid regions with drought stress. Vegetable amaranth is the inexpensive sources of natural antioxidants like, vitamins, phenolics, flavonoids and a unique source of betalain (betacyanin and betaxanthin). These secondary metabolites or natural antioxidants are involved in defense against several diseases like cancer, atherosclerosis, arthritis, cataracts, emphysema, and retinopathy, neuro-degenerative and cardiovascular diseases [35, 48]. Drought stress leads to the accumulation of reactive oxygen species (ROS), which might initiate destructive oxidative processes such as lipid peroxidation, chlorophyll and betalain bleaching and protein oxidation. Plants have evolved both enzymatic and non-enzymatic defense systems for scavenging and detoxifying ROS, resulting in antioxidant defense capacity [78]. Drought ameliorates active accumulation of solutes (e.g., proline, α-tocopherol and polyphenol) to protect them against oxidative damage and allows plants to maintain positive turgor pressure, a requirement for maintaining stomata aperture and gas exchange [79]. Besides, non-enzymatic antioxidants like, leaf pigments, ascorbic acid, carotenoids, phenolics and flavonoids have a protective role to avoid ROS generation [80].

Thus, there are three general types of response to drought stress including [81]: a) mechanisms to avoid water loss (*e.g.* osmotic adjustment), b) mechanisms for protection of

cellular components (*e.g.* qualitative and quantitative changes of pigments), and c) mechanisms of repairing against oxidative damage (*e.g.* antioxidant systems).

Excessive accumulation of reactive oxygen species (hydrogen peroxide, H_2O_2; superoxide, $O_2^{\bullet-}$; hydroxyl radical, OH• and singlet oxygen, $^1O_2$), and malondialdehyde are enhanced under abiotic and/or biotic stresses, which can cause oxidative damage to plant macromolecules and cell structures, leading to inhibition of plant growth and development, or to death. Among the various ROS, freely diffusible and relatively long-lived H_2O_2 acts as a central player in stress signal transduction pathways. These pathways can then activate multiple acclamatory responses that reinforce resistance to various abiotic and biotic stressors. To utilize H_2O_2 as a signaling molecule, non-toxic levels must be maintained in a delicate balancing act between H_2O_2 production and scavenging.

Amaranthus tricolor is often described as drought tolerant plants [68]. There are few reports related to the effect of drought stress on secondary metabolites of different crops including leafy vegetables. There is no information in *Amaranthus tricolor* for chlorophyll, ROS markers like, lipid peroxidation, H_2O_2, electrolyte leakage, compatible solutes and non-enzymatic antioxidants like, proline, total carotenoid, reduced ascorbic acid, soluble protein, phenolics, flavonoids and total antioxidant activity under drought stress, although majority of these phytochemicals have recently attracted attention for their antioxidant activities. In our previous studies [143, 149-151, 160-162, 173] we selected some antioxidant enrich high yielding cultivars. Therefore, present investigations were aimed elucidate key mechanisms involved in drought tolerance by comparing selected *A. tricolor* cultivars, differing in their extent of drought tolerance, (ii) to identify tolerant cultivars to drought stress, (iii) to explore the relationships among physiological, ROS markers, compatible solutes and non-enzymatic antioxidant to obtain more tolerant cultivars under drought stress.

Materials and methods

Plant materials and experimental conditions

We selected four antioxidant enrich high yielding cultivars of *A. tricolor* from 102 genotypes collected in different echo-geographical regions of Bangladesh on the basis of our earlier studies [143, 149-151, 160-162, 173]. Accession number of these four cultivars were VA6, VA11, VA14 and VA16. Four *Amaranthus tricolor* cultivars were grown in pots under rain shelter open field of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh (AEZ-28, $24^0 23'$ north latitude, $90^0 08'$ east longitude, 8.4 m.s.l.). This facility moves over the trial area during rainfall events and otherwise exposes plants to ambient field

conditions. The pot soil was collected from the topsoil layer of experimental station (30 cm depth). The soil was silty clay with slightly acidic (pH 6.4) and low in organic matter (0.87%), total N (0.09%) and exchangeable K (0.13 c mol kg^{-1}). The soil S content was at par with critical level, while P and Zn contents were above the critical level (Critical levels of P, S and Zn were 14, 14 and 0.2 mg kg^{-1}, respectively and that of K was 0.2 c mol kg^{-1}). The seeds were sown on 1st March 2016, in plastic pots of 22 cm in height and 40 cm in diameter (upper side) in 5 cm apart rows. Randomized complete block design (RCBD) pattern with three replications was adopted for the experiment. Total 48 pots were sown with 12 pots per genotypes and 12 pots per treatment. Fertilizer was applied at the rate of 92:48:60 kg ha^{-1} N:P_2O_5:K_2O as split dose. First at upper 15 cm of pot soil at the rate of 46:48:60 kg ha^{-1} N: P_2O_5:K_2O and second at 10 DAS at the rate of 46:0:0 kg ha^{-1} N: P_2O_5:K_2O. The average day/night temperatures, relative humidity and day length during the experimental period was 26/22 °C, 75%, and 12 h, respectively. Each cultivar was grouped into three sets and subjected to four water stress treatments that is, severe drought stress (SDS) or 25% field capacity (FC), moderate drought stress (MDS) or 50% FC, low water drought (LDS) or 80% FC, and control or 100% FC. Pots were well irrigated everyday up to 10 days after sowing of seeds for proper establishment and vigorous growth of seedlings. Imposition of water stress treatment was started at 25 DAS. Each pot was weighed twice a day at 12 h intervals and the amount of water equaling that lost through transpiration and soil evaporation was added to achieve the target field capacity of each water condition. Imposition of water stress was continued up to 55 DAS. At 55 DAS the leaves of *Amaranthus tricolor* was harvested. Sampling was done around midday between 11:00 and 12:00 h from the top fully emerged young leaves from control and stressed plants for quantifying the plant parameters. All the parameters were measured in three replicates.

Plant growth measurements

At 55 DAS, 5 plants were sampled for total biomass and specific leaf area measurement. Total leaf area per plant was measured with a LI-3100 leaf area meter (LICOR. Inc., Lincon, NE, USA). Dry mass of total plant and leaves was obtained after oven drying the samples at 70 °C until constant weight achieved. Specific leaf area (SLA) was calculated as total plant leaf area divided by the leaf dry weight.

Determination of leaf relative water content

Leaf relative water content (RWC) was measured according to the method of Ogbaga [182]. For determination of leaf relative water content (RWC), fully expanded leaves of three plants per replicate were used. Three leaf discs (10 mm in diameter) were punched from the interveinal area of each plant using a cork borer and the fresh mass (FW) of pooled discs per

replicate was determined immediately. Weighed leaf discs were then placed in distilled water for 4 hours at 20 °C under dim illumination to avoid respiratory losses. Four hours of floating in water was found to be sufficient for complete hydration of leaf discs. The leaf discs were then carefully blotted to remove surface water and turgid mass (TW) was taken to calculate water uptake. Dry mass (DW) of the leaf discs was determined by drying the tissues at 70 °C for 2 to 4 d. RWC was calculated as (FW - DW)/ (TW - DW) × 100.

Determination of chlorophyll and total carotenoid content

Chlorophyll *a*, chlorophyll *b*, chlorophyll *ab* and total carotenoid was determined following the procedure described in the previous chapter

Oxidative stress markers

Determination of leaf malondialdehyde and H_2O_2

Malondialdehyde (MDA) was measured using 2-thiobarbituric acid (TBA) according to Zhao *et al.* [183]. Briefly, 1 g of fresh vegetable amaranth leaf was ground with 5 ml 0.6 % TBA in 10 % trichloroacetic acid (TCA), using a mortar and pestle. Then, the mixture was heated at 100 °C for 15 min. After cooling the mixture in ice, it was centrifuged at 5000 rpm/min for 10 min. The absorbance of the supernatant was read at 450, 532, and 600 nm. The MDA content was calculated on a fresh weight basis as follows:

μmol MDA g^{-1} FW = 6.45 (OD532−OD600) – 0.56OD450 and finally, data were expressed as nmoles per gram fresh weight (nmol g^{-1} FW). Hydrogen peroxide was measured after reaction with KI. The reaction mixture consisted of 0.5 ml 0.1%, trichloroacetic acid (TCA) leaf extract supernatant, 0.5 ml of 100 mM potassium phosphate buffer, and 2 ml reagent (1 ml KI w/v double-distilled water). The blank probe consisted of 0.1 % TCA in the absence of leaf extract. The reaction was developed for 1 h in the dark, and absorbance was determined at 390 nm. The amount of hydrogen peroxide was measured according to standard curve that was prepared with known concentrations of H_2O_2 and data were expressed as μmoles per gram fresh weight (μmol g^{-1} FW).

Determination of electrolyte leakage

Electrolyte leakage (EL) was determined as described by Lutts et al. [184]. Six randomly chosen plants per treatment (four mature leaves per plant) were taken and cut into 1cm segments. Leaf samples were washed three times with distilled water to remove surface contamination, and then placed in individual stoppered vials containing 10 mL of distilled water. The samples were incubated at room temperature (25 °C) on a shaker (100 rpm) for 24 h. Electrical conductivity of the bathing solution (EC1) was read after incubation. The same samples were then placed in an autoclave at 120 °C for 20 min and a second reading of the EC

(EC2) was made after cooling the solution to room temperature. The EL was calculated as EC1/EC2 and expressed as percentage.

Compatible solutes

Determination of leaf proline content

Proline was assayed from freeze dried leave material, using a 3% sulfosalicylic and ninhydrin extraction buffers according to Bates *et al*. [185]. Samples of 0.04 g dry weight of leaves was homogenized with 3% (w/v) sulfosalicylic acid and centrifuged at 3000 g for 10 min. A 200 µl aliquot of the supernatant was mixed with 400 µl of the reagent mixture (30 ml glacial acetic acid, 20 ml phosphoric acid and 1.25 g ninhydrin) and heated in sealed test tubes at 100 °C for 1 h. After cooling down, 4 ml toluene was added to each sample. Proline content was measured on a spectrophotometer (Hitachi, U-1800, Tokyo, Japan) at 520 nm and expressed as µmoles per gram dry weight (µmol g^{-1} DW).

Determination of soluble protein content

Soluble proteins were determined by spectrophotometry at 595 nm, applying the dye-binding method and bovine serum albumin as standard [186].

Non-enzymatic antioxidants

Determination of free ascorbic acid

Free (reduced) ascorbic acid in amaranth leaves was quantified according to the procedure described by Ma *et al*. [187] with slight modifications. Dry leaves powder (0.5 g) was homogenized in 8 ml 5% (w/v) TCA on ice, centrifuged at 10,000 g for 10 min at 4°C, and the supernatant was used immediately for analysis. Then 0.8 ml supernatant was added to a reaction mixture containing 1 ml 10% (w/v) TCA, 800 µl 42% (w/v) ortho-phosphoric acid, 800 µl 65 mM 2, 2-dipyridyl in 70% (v/v) ethanol, and 400 µl 3% (w/v) ferric chloride. The reaction was incubated at 42 °C for 1 h, and the absorbance was measured at 525 nm. Free ascorbic acid content was determined based on a standard curve generated with known ASA concentrations.

Extraction of samples for TPC, TFC and TAC analysis

Samples were extracted following the procedure described in the previous chapter

Determination of TPC, TFC and TAC

TPC, TFC and TAC were measured following the procedure described in the previous chapter

Statistical Analysis

The results were reported as the mean ± SD of three separate measurements. The data were also statistically analyzed by the ANOVA program in Statistix 8 software, and the means were

compared by the Duncan's multiple range (DMRT) test at 1% level of probability. Microsoft Excel program was used to present the figures.

Results and discussion

The results showed significant difference ($P<0.01$) for all the traits across drought stresses.

In the present study, several adaptive responses were observed in *Amaranthus tricolor* cultivars under four water deficit conditions. Tested cultivars exhibited some morphological, physiological and biochemical changes under different levels of drought. The tested cultivars had tremendous variability among studied ROS markers, compatible solutes and non-enzymatic antioxidant parameters. Among studied cultivars, VA14 and VA16 had higher biomass, SLA, chlorophyll content, RWC, compatible solutes and non-enzymatic antioxidant like, total carotenoids, free ascorbic acid, proline, TPC, TFC and TAC and lower oxidative stress responses due to less accumulation of stress markers like, MDA, H_2O_2 and EL related to higher water use efficiency could be identified as a drought tolerant cultivar. These two cultivars could be used as drought tolerant cultivars or selected as tolerant parents to obtain more tolerant cultivar in hybridization programs.

Total biomass and specific leaf area (SLA)

Moderate drought stress (MDS) and severe drought stress (SDS) resulted a significant reduction in total biomass for all cultivars. Nevertheless, cultivars VA14, VA16 had higher total biomass across all drought treatments compared to cultivar VA6 and VA11. Total biomass was sharply declined with severity of water deficit treatments in all cultivars. VA6 exhibited the highest reduction of total biomass (48.88 and 61.38% at MDS and SDS, respectively) whereas, the lowest reduction of total biomass was noted in VA14 (27.84 and 47.74% at MDS and SDS, respectively) (Fig. 1a). Specific leaf area (SLA), an indicator of leaf thickness gradually decreased with increasing drought severity, however, reductions were higher in VA11 (14.11 and 20.20% at MDS and SDS, respectively) and VA6 (11.43 and 18.20% at MDS and SDS, respectively) (Fig. 1b).

Growth (biomass production) are the primary processes to be affected by drought [85]. In our investigation, growth reduction was observed under moderate and severe stress. This suggests that even at reduced soil water availability, *Amaranthus tricolor* cultivars are able to grow. Our results agree with the findings of Achten *et al.* [188] in *J. curcas* who have observed that water withhold would arrest growth but maintaining plants at low soil water availability (40%) would allow them to continue growing, although at a slower rate than fully irrigated condition. Total biomass of all *A. tricolor* cultivars significantly reduced when exposed to

drought stress, in terms of drought severity dependent manner, which indicated that drought stress depressed plant growth. Reduced biomass under severity of drought treatment could be attributed by inhibition of cell elongation and expansion, reduced turgor pressure, alteration of energy from growth to synthesis of compatible solutes to maintain cell turgor reduced water uptake resulting in a decrease in tissue water contents and trimming down the photo-assimilation and metabolites required for cell division [189]. VA14 and VA16 had less growth inhibition and the highest sustainability in production compared to other cultivars under drought stress which suggested tolerance to drought. Our study demonstrated that SLA, an indicator of leaf thickness, had a significant decrease to the severity of drought stress in all cultivars. The reduction was observed under moderate and severe stress. This suggests that even at reduced soil water availability, *Amaranthus tricolor* cultivars are able to maintain SLA. Similar trend of decline in SLA was observed by Guerfel *et al.* [190]. VA14 and VA16 exhibited higher SLA compared with other cultivars, suggesting the better performance to accumulate more dry mass per unit of leaf area under drought stress. We observed the positive relationship between SLA and total biomass (r = 0.86** Supplemental Table S1), suggested that salinity may also influence plant growth through reduction in specific leaf area.

Leaf relative water content (RWC)

Cultivar VA14 had the highest leaf RWC values followed by VA16, while VA6 exhibited the lowest leaf RWC followed by VA11 for all water deficit treatments. Leaf RWC gradually decreased with increasing drought severity, however, reductions were higher in VA6 (12.71 and 21.18% at MDS and SDS, respectively) and VA11 (11.45 and 19.87% at MDS and SDS, respectively) whereas, it was the lowest in VA14 (0.32 and 4.99% at MDS and SDS, respectively) (Fig. 1c). Relative water content is useful variables to evaluate the physiological water status of plants and its metabolic activity and survival that could be used as an attribute for discriminating tolerant and sensitive plants under water deficit [191]. *Amaranthus tricolor* cultivars established drought-induced reduction of RWC with the severity of drought ($P < 0.01$). RWC reduction was observed under moderate and severe stress. This suggests that even at reduced soil water availability, *Amaranthus tricolor* cultivars are able to maintain RWC. Reduced turgor pressure, reduced water uptake due to shortage of available water in the soil caused by drought, hinder water uptake by roots, resulting in decrease of RWC in the leaves. In this investigation, a highly negative correlation between RWC and both proline and soluble protein contents was observed (r = -0.63**, r = -0.55* Supplemental Table S1). It has also been extensively documented in several species that compatible solutes like free proline and soluble protein accumulation facilitate osmoregulation under drought stress [192]. VA14 and VA16

exhibited the higher leaf RWC under drought stress conditions, could be elucidated by a potential osmoregulation strategy due to the higher accumulation of compatible solutes in comparison to another cultivar studied. Thus, these two cultivars seem to be more efficient in terms of decreasing the cellular osmotic potential allowing the roots to absorb a sufficient amount of water to maintain cell turgidity and for improving potentiality in hydration status. chlorophyll *b* and chlorophyll *ab* in VA6. VA11, VA14 and VA16 were (38%, 11%, 39% and 20%); (15%, 32%, 48% and 50%) and (31%, 19%, 43% and 30%), respectively (Fig. 1d, 1e & 1f). Decline of chlorophyll *a*, *b* & *ab* was lesser from control to MDS. Reduction of chlorophyll *b* was higher than chlorophyll *a*. The highest chlorophyll *a*, *ab* was observed in VA14 followed by VA16 while, the highest chlorophyll *b* was recorded in VA16 followed by VA14 and the lowest chlorophyll *a*, *b* & *ab* were detected in VA6 under control and LDS condition. Although VA14 showed the highest chlorophyll *a*, & *ab* under control and LDS condition, but due to its

Photosynthetic pigment content

Soil water deficit gradually reduced chlorophyll *a*, *b* and *ab* in all cultivars of vegetable amaranth in following order: control > LDS > MDS. In SDS, all types of chlorophyll contents were sharply declined in all varieties. From MDS to SDS, reduction of chlorophyll *a,* dramatic reduction, VA16 had the highest chlorophyll *a* & *ab* under SDS. Across the varieties, VA11 exhibited the highest reduction in chlorophyll *a*, *b* & *ab* under MDS and SDS (Fig. 1d, 1e & 1f).

Inhibition of lipid peroxidation, H_2O_2 accumulation and electrolyte leakage (EL)

In the present study, the effect of drought stress was assessed on MDA and H_2O_2 production. In response to drought stress from control to LDS, there was no significant increase in MDA and H_2O_2 whereas, significant sharp increase in MDA and H_2O_2 was noticed from MDS to SDS. The lowest MDA and H_2O_2 was found in VA16 followed by VA14. While, the highest MDA and H_2O_2 was observed in VA6, followed by VA11 ($P < 0.01$)). VA6 exhibited 0.47 and 0.89-fold increase of MDA, 0.46 and 1.36-fold increase of H_2O_2 at MDS and SDS, respectively. In contrast, VA16 had 0.89 and 0.88-fold increase of MDA, 1.32 and 1.82-fold increase of H_2O_2 under MDS and SDS, respectively (Fig. 2a & 2b). There was no significant difference of EL among the cultivars under control treatment, however, all the cultivars were significantly increased with the severity of drought stress from LDS to SDS. The lowest EL was observed in VA14 followed by VA16, while VA6 exhibited the highest EL followed by VA11 from LDS to SDS drought treatments. EL sharply increased with severity of drought, nevertheless, increments were the lowest in VA14 (0.22, 0.90 and 2.06 fold, for LDS, MDS and SDS,

respectively) and the highest in VA6 (0.27, 3.96 and 5.28 fold for LDS, MDS and SDS, respectively) (Fig. 2c). There was a significant difference in photosynthetic leaf pigment

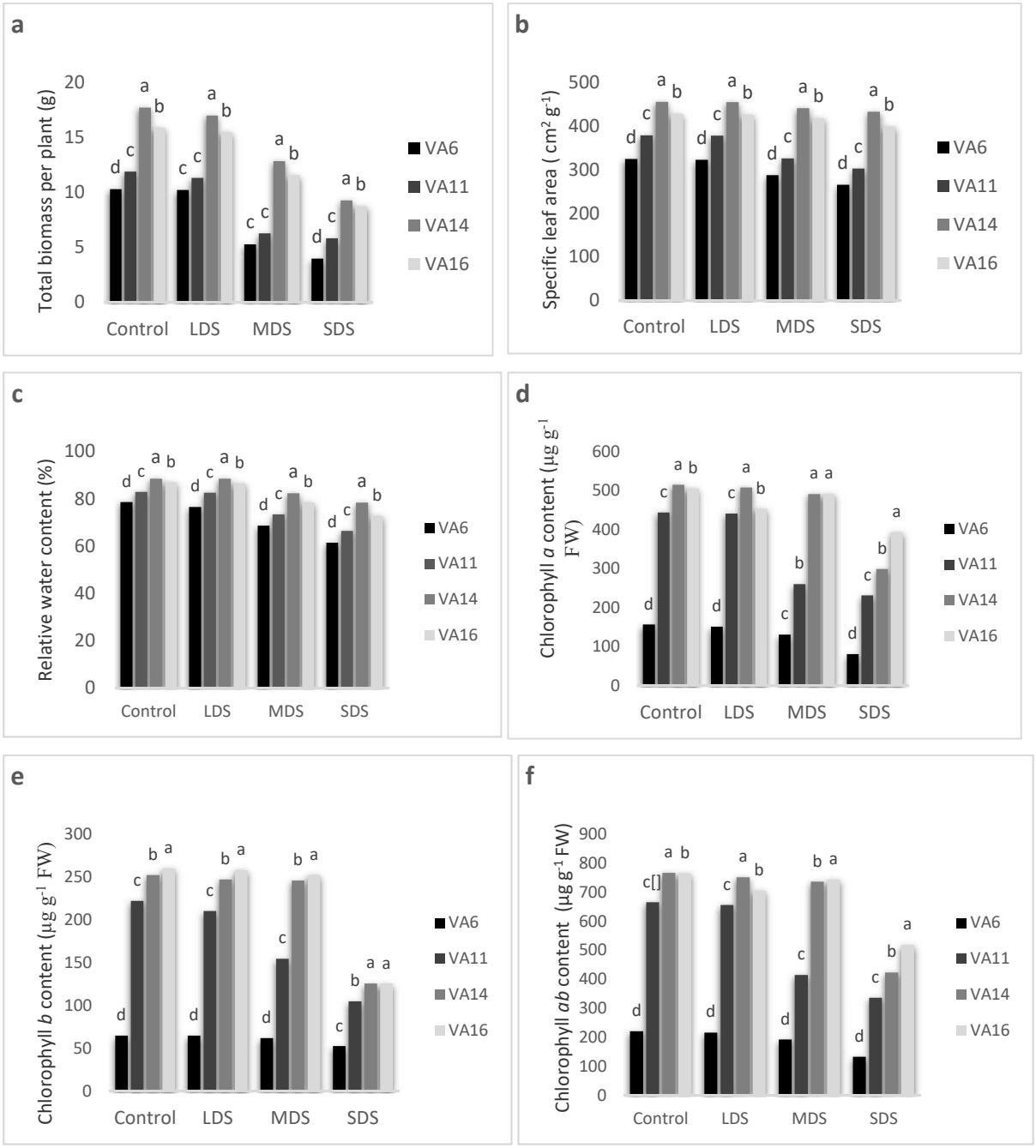

Fig. 1. Response of growth, leaf water content and photosynthetic pigments [a, Total biomass plant^{-1} (dry) (g); b, Specific leaf area (cm^2 g^{-1}); c, Relative water content (%); d, Chlorophyll *a* (µg g^{-1} FW); e, Chlorophyll *b* (µg g^{-1} FW); f, Chlorophyll *ab* (µg g^{-1} FW)] in selected *Amaranthus tricolor* cultivars under four irrigation regimes: Control (100% FC), LDS (80% FC), MDS (50% FC), and SDS (25% FC), (n = 3, P < 0.01)

(chlorophyll *a*, *b* & *ab*) ($p < 0.01$) among all soil water deficit treatments and among all amaranth varieties. An increase in water deficit stress inhibiting chlorophyll synthesis which is supposed to occur at four consecutive stages: (I) the formation of 5-aminole-vuliniuc acid (ALA); (II) ALA condensation into porphobilinogen and primary tetrapyrrol, which is

transformed into protochlorophyllide; (III) light-dependent conversion of protochlorophyllide into chlorophyllide; and (IV) synthesis of chlorophylls *a* and *b* along with their inclusion into under drought stress, also associated to free radical-induced oxidation of chlorophyll pigment developing pigment–protein complexes of the photosynthetic apparatus [193]. The observed decrease in photosynthetic leaf pigment [194], disruption of some chloroplasts or a consequence of increased activity of chlorophyll degrading enzyme, chlorophyllase [195]. Lutts *et al*. [196] indicated that chlorophyll concentration in stressed tissues can be construed as an index of tissue tolerance to drought. In this study, VA16 and VA14 having more chlorophyll content than the other studied cultivars, it could be suggested that these cultivars are more drought tolerant than the others. The reduction of photosynthetic leaf pigment was found lower in all cultivars, this may be due to present of antioxidant leaf pigment betalain (Betacyanin and betaxanthin) that absorbed significant amount of radiation and protected the drought stressed chloroplasts from harmful excessive light. These results were fully agreement with the results of Jain *et al.* [197]. They found that high betalain content in *Disphyma australe* showed physiologically more tolerant to salt stress. Moreover, betalain protects the drought stressed chloroplasts by reducing the ROS in thailakoids [198].

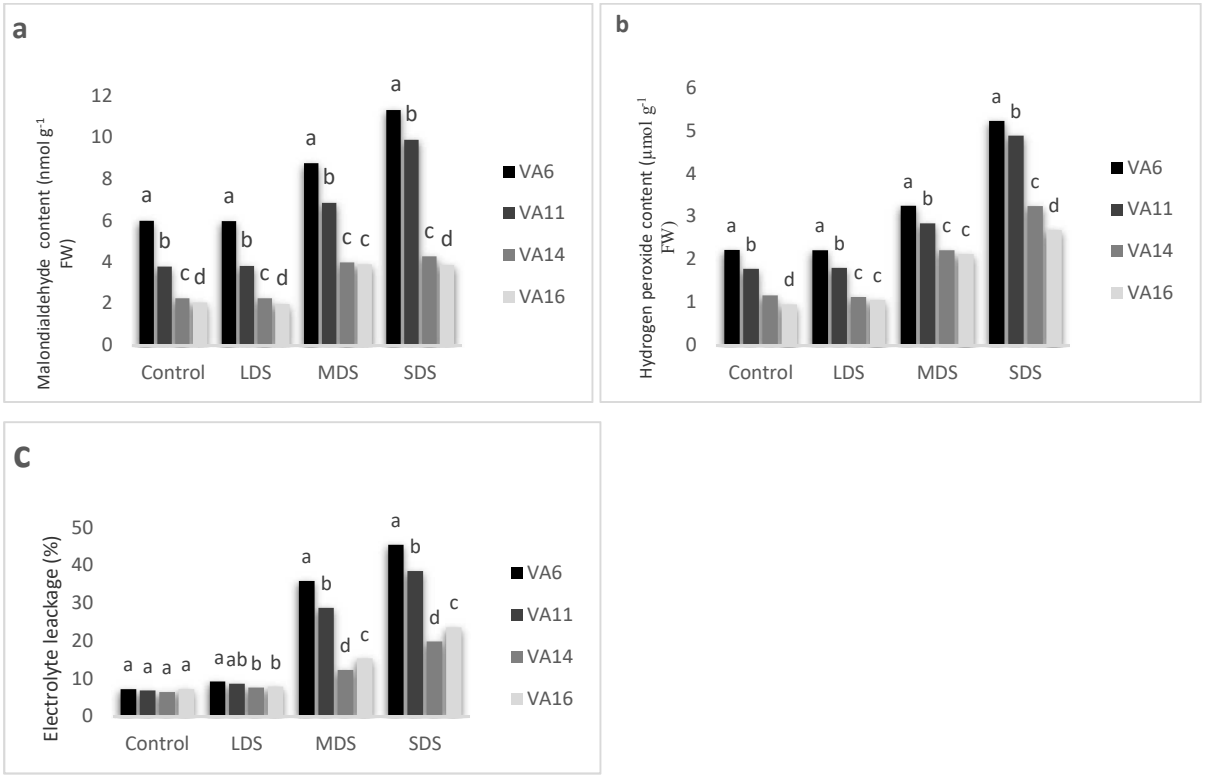

Fig. 2. Changes of ROS markers [a, Malondialdehyde (MDA) content (nmol g^{-1} FW); b, Hydrogen peroxide (H_2O_2) content (µmol g^{-1} FW); c, Electrolyte leakage (%)] in selected *Amaranthus tricolor* cultivars under four irrigation regimes: Control (100% FC), LDS (80% FC), MDS (50% FC), and SDS (25% FC), (n = 3, P < 0.01)

Drought stresses aggravate the production of ROS like superoxide, hydrogen peroxide, hydroxyl radicals, alkoxy radicals, singlet oxygen etc. resulting in oxidative damage in cell [199]. Mechanisms of ROS generation in biological systems are electron reduction (O_2) at higher oxygen concentrations, initial activation of O_2 by xanthine oxidase, dis-mutation of the superoxide anion by superoxide dismutase to yield H_2O_2. ROS may react with proteins, lipids and DNA, causing oxidative damage and impairing the normal functions of cells. Various organelles including chloroplasts, mitochondria and peroxisomes are the seats as well as first target of ROS produced under drought stress [189]. In MDS and SDS, both MDA and H_2O_2 content was remarkably increased that agreed with the results those observed in strawberry [200]. In the present investigation, extreme accumulation of H_2O_2 in MDS and SDS might have accelerated the Haber-Weiss reaction, resulting in hydroxyl radical (OH•) formation and therefore, resulting in serious lipid peroxidation and cell membrane damage [103]. The static ROS content from control to LDS might be due to inhibition of ROS generation in plant tissues and up-regulates ROS scavenging activity by active accumulation of excessive proline, total carotenoid, ascorbic acid, TPC, TFC and antioxidant activity that inhibited the increment of MDA and H_2O_2 content. Although the highest accumulation of proline, total carotenoid, ascorbic acid, TPC, TFC, antioxidant activity was noted in SDS condition compared to any stresses, but MDA and H_2O_2 accumulation was also the highest. This might be likely that the vegetable amaranth fell in to severe stress and could not cope with damage caused by drought. Maintaining a balance between ROS production and scavenging is crucial under stressed conditions [201]. In our study, compatible solutes and non-enzymatic antioxidants like proline, total carotenoid, ascorbic acid, TPC, TFC and antioxidant activity was significantly increased as a protective mechanism under drought-stressed conditions from LDS to SDS to reduce the H_2O_2 and MDA accumulation. The exposure of four cultivars in MDS and SDS exhibited differential increment of H_2O_2 and MDA accumulation, this might be due to the differential responses of ROS (H_2O_2, MDA) scavenging ability of these cultivars. Under MDS and SDS, less tolerant cultivar VA6 showed the highest H_2O_2 and MDA content showing that this cultivar experienced more lipid peroxidation and higher levels of cellular damage (Fig. 2a, 2b). This cultivar also had the lowest compatible solutes and non-enzymatic antioxidant like proline total carotenoid, ascorbic acid, TPC TFC and antioxidant activity compared to any cultivars. In contrast, tolerant cultivars VA16 and VA14 had low H_2O_2 and MDA content and alleviated the oxidative stress through transcriptional regulation of multiple defense pathways, such as compatible solutes and non-enzymatic antioxidant, antioxidant enzymes and the ASC-GSH cycle and improved the effects caused by drought stress through protecting ROS biosynthesis.

(See compatible solute accumulation and non-enzymatic antioxidant section). Enhanced electrolyte leakage is considered to be a sign of destruction and deterioration under water stress. In this study, electrolyte leakage was remarkably increased with the drought severity. It indicated that cell electrolyte leakage could be used as a criterion to differentiate stress tolerant and susceptible cultivars and that in some cases lower electrolyte leakage could be correlated with abiotic stress tolerance. Furthermore, the observed dramatic increase in MDA and H_2O_2 under drought severity induced cellular membrane damage, which is demonstrated by an increase in EL. VA14 and VA16 showed lower electrolyte leakage can be used as tolerant cultivars. Moreover, MDA H_2O_2 and EL showed the strong negative correlation observed with total biomass (r = -0.91**, r = -0.89** and -0.79** Supplemental Table S1) suggests that the drought induced lipid peroxidation and H_2O_2 generation oxidative stress can be one of the reasons for inhibition of biomass production in *A. tricolor* plants.

Impact of drought on compatible solute accumulation

One of the most common stress tolerance strategies in plants is the overproduction of different types of compatible organic solutes. Generally, they protect plants from stress through different means such as contribution towards osmotic adjustment, detoxification of ROS, stabilization of membranes, and native structures of enzymes and proteins [189]. In this study, the proline content of the leaves had significant ($p < 0.01$) and remarkable increase across all cultivars under all drought stresses. Highly significant negative correlations between the proline and soluble protein content in *Amaranthus tricolor* leaves and the biomass production (r = -0.66** and -0.61**, respectively), were observed (Supplemental Table S1). The highest proline accumulation in response to drought stress observed in VA14 and VA16 might be related to their competitive ability in a drought against oxidative stress. Proline have antioxidant activity, activates detoxification systems, contributes to cellular homeostasis by protecting the redox balance, and functions as protein precursor, an energy source for the stress recovery process (See ROS markers section). It mainly involved in protection against oxidative stress thus reduced lipid peroxidation resulting in in different plant species and had an essential role in stabilizing proteins and cellular membranes in plant cells in presence of high levels of osmolytes. In addition, proline induces expression of stress-induced responsive genes, activates antioxidant enzymes [202]. Proline protects photosynthetic apparatus. In our study, proline accumulation is high that resulted in less decline of chlorophyll content (see photosynthetic leaf pigment section). Synthesis of stress proteins is a ubiquitous response to cope with prevailing stressful conditions including water deficit. Most of the stress proteins are soluble in water and therefore contribute towards the stress tolerance phenomena by hydration of

cellular structures [189]. In our study, soluble protein didn't have any role in *A. tricolor* cultivar except of susceptible cultivar VA6 that had increasing trend of soluble protein.

The proline content of leaves had significant ($p < 0.01$) and dramatic increase across all varieties under all drought stresses in following order: control < LDS < MDS < SDS (Fig. 3a). In control condition, the proline content was low (22.26, 27.88, 40.26 and 34.85 µmol g^{-1}, in VA6, VA11, VA14 and VA16, respectively), however, under MDS and SDS, the proline content of amaranth was increased approximately 3fold under MDS and more than 4fold under SDS compared to control condition and reached to 90.46, 116.51, 154.55 and 149.26 µmol g^{-1}, in VA6, VA11, VA14 and VA16, respectively. In all water stresses from control to SDS, the highest proline content was observed in VA14 (around 2fold compared to VA6) followed by VA16 while, the lowest proline content was noticed in VA6 under all water deficit conditions (Fig. 3a). Four cultivars respond inconsistently with severity of drought. In VA6, the soluble protein content was elevated to 9.00%, 16.07% and 34.20% at LDS, MDS and SDS, respectively, as compared to control condition. While, soluble protein of VA11 and VA16 was static from control to MDS and slightly increased (15.50%) and remarkably decreased (44.08%), respectively under SDS compared to control condition. Drought stress caused remarkable reduction of soluble protein in VA14 under MDS (2.73%) and SDS (38.39%) (Fig 3b).

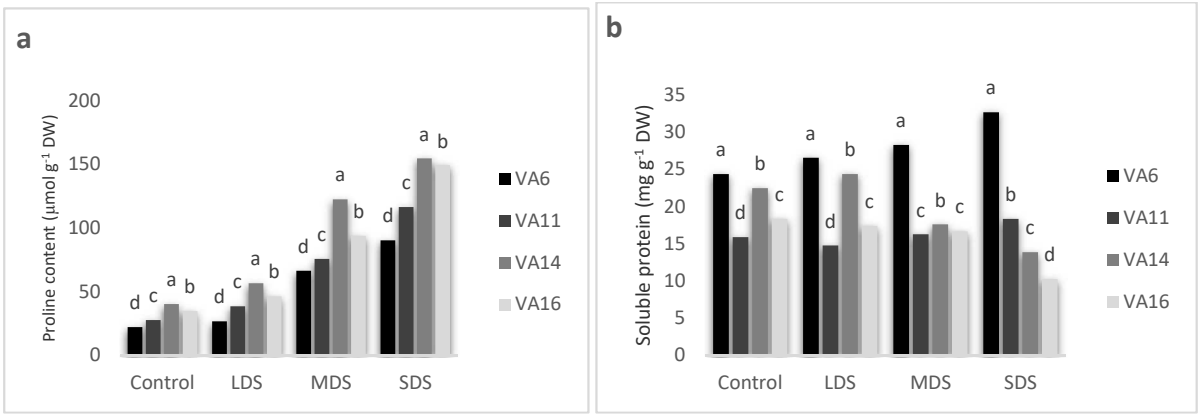

Fig. 3. Accumulation of compatible solutes [a, Proline content (µmol g^{-1} DW); b, Soluble protein content (mg g^{-1} DW)] in selected *Amaranthus tricolor* cultivars under four irrigation regimes: Control (100% FC), LDS (80% FC), MDS (50% FC), and SDS (25% FC), (n = 3, P < 0.01)

Non-enzymatic antioxidant

The highest total carotenoid was observed in VA16 and the lowest was noted in VA11 while, the highest ascorbic acid was observed in VA14 and the lowest was noted in VA6 under all water treatment conditions. VA16 exhibited 0.31 and 1.02-fold increase of total carotenoid, nevertheless, VA11 showed 0.31 and 0.52-fold increase of total carotenoid under MDS and

SDS, respectively. In contrast, VA14 had 1.34 and 2.42-fold increase of ascorbic acid and VA6 exhibited 0. 23 and 0.77-fold increase of ascorbic acid under MDS and SDS, respectively (Fig. 4a & 4b).

Increment of total polyphenol (TPC) is depended on degree of water stress (Fig 4c). Remarkable and sharp increase of polyphenol content was exhibited at MDS (30.08%, 80.93%. 32.74%, and 15.94% in VA6, VA16, VA11 and VA14, respectively) and under SDS (169%, 142%, 75% and 39% in VA6, VA16, VA11 and VA14, respectively). TFC was significantly and moderately elevated with severity of drought stress from control to SDS in following order: control < LDS < MDS < SDS (Table 2). VA14 had the highest TFC over all drought levels. In contrast, the lowest TFC was recorded in VA6 from control to LDS and VA16 from MDS to SDS. Flavonoid content moderately increased under MDS (3.48%, 25.27%, 16.12% and 11.78% in VA6, VA16, VA11 and VA14, respectively) and at SDS (30%, 25%, 42% and 22% in VA6, VA16, VA11 and VA14, respectively) compared to control condition (Fig 4d). Carotenoids have received little attention despite their capacity to scavenge singlet oxygen and lipid peroxy-radicals, as well as to inhibit lipid peroxidation and superoxide generation under dehydrative forces. A major protective role of carotenoids and beta-carotene in photosynthetic tissue may be through direct quenching of triplet chlorophyll, which prevents the generation of singlet oxygen and protects from oxidative damage and help plants to withstand adversaries of drought [189]. Total carotenoid is a lipophilic antioxidant and are able to detoxify various forms ROS [203]. Plants are able to release of excessive energy by thermal dissipation associated with an increase in the total carotenoid concentration in water stressed plants. This can be attributed to the activation of the xanthophyll cycle. Thus, presumed that the role of antioxidants and beta-carotene pigment in regulating photosynthetic electron transport is crucial [204]. Ascorbic acid (AA) is one of the powerful antioxidants [205]. Ascorbic acid along with vitamin E plays a key role in quenching intermediate/excited reactive forms of molecular oxygen either directly or through enzymatic catalysis. It allows enzymatic and non-enzymatic antioxidant defense system and thereby increased efficiency and contribution to ROS neutralization and balance. AA can directly scavenge superoxide, hydroxyl radicals and singlet oxygen and diminish H_2O_2 to water via ascorbate peroxidase reaction [206]. Recently, dehydroascorbic acid has emerged as a signaling molecule regulating stomatal closure [207]. In this study, both total carotenoid and ascorbic acid had significant and remarkable ($p < 0.01$) increment across drought stresses and cultivars. These results were fully agreed with the results of Choysum in dry season trial by Hanson *et al*. [74], where, they found the elevated response of total carotenoid and ascorbic acid, respectively from control to drought stress. The highest

total carotenoid and ascorbic acid were observed in VA16 and VA14, respectively under all water treatment conditions while, VA6 and VA11 exhibited the lowest total carotenoid and ascorbic acid, respectively (Fig. 4a, 4b). The increased content of ascorbic acid, indicates the crucial role of the ASC–GSH cycle for scavenging ROS in leaves of *A. tricolor*. Similarly, the drought tolerant, but not the sensitive cultivar, accumulated higher activities and transcripts of the ASC–GSH cycle.

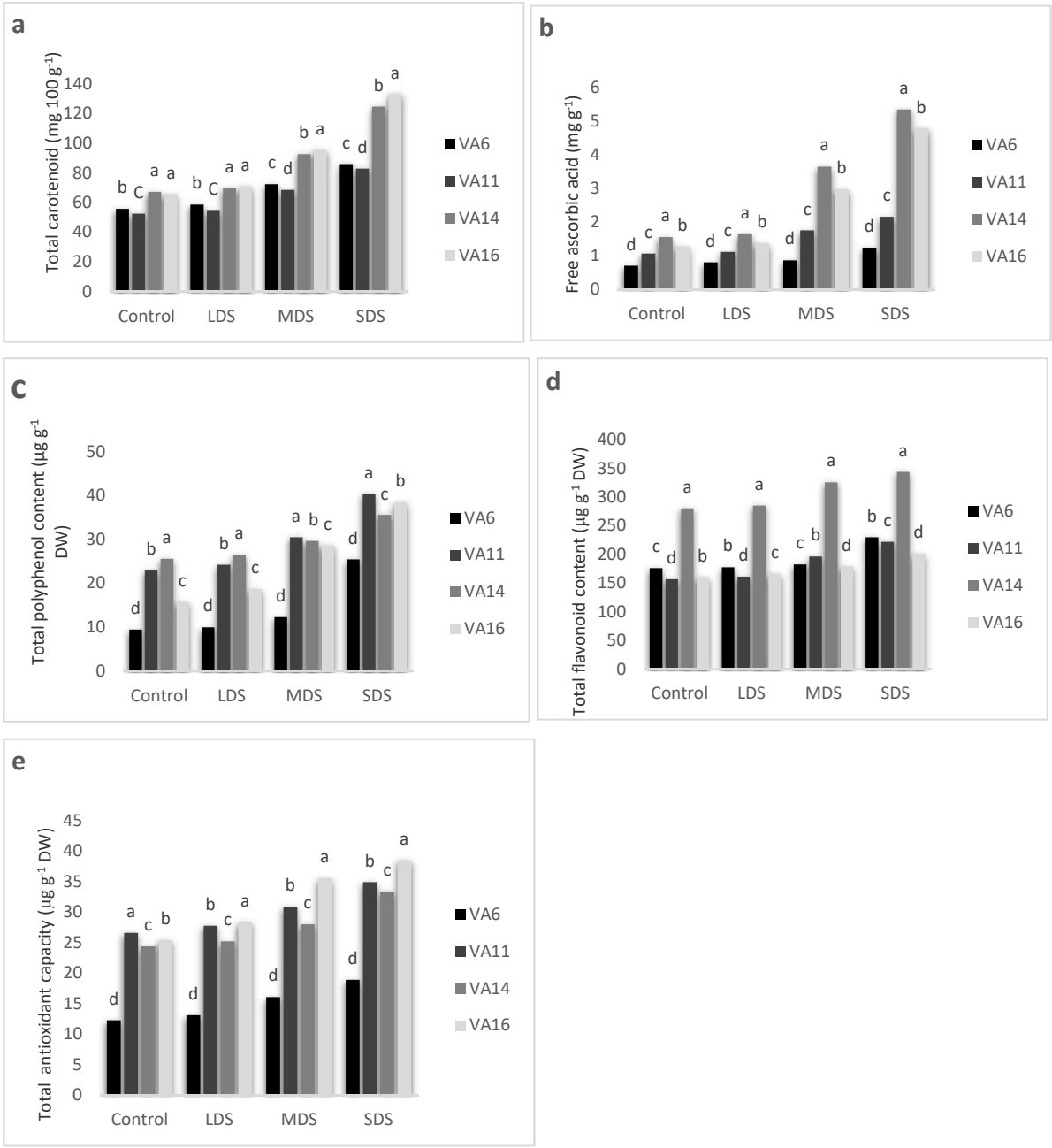

Fig. 4. Influence of non-enzymatic antioxidants [a, Total carotenoid content (mg 100 g^{-1} FW); b, Free ascorbic acid content (mg g^{-1} FW); c, total polyphenol content (GAE µg g^{-1} DW); d, Total flavonoid content (RE µg g^{-1} DW), e, DPPH radical scavenging activity (TEAC µg g^{-1} DW)] in selected *Amaranthus tricolor* cultivars under four irrigation regimes: Control (100% FC), LDS (80% FC), MDS (50% FC), and SDS (25% FC), (n = 3, P < 0.01)

Polyphenol and flavonoid

Generally, accumulation of polyphenols which possess antioxidant properties is stimulated in response of ROS increases under biotic and abiotic stresses. They are plentiful present in plant tissues [205]. Polyphenols can chelate transition metal ions, can directly scavenge molecular species of active oxygen, and may quench lipid peroxidation by trapping the lipid alkoxyl radical. Furthermore, flavonoids and phenylpropanoids are oxidized by peroxidase, and act in H_2O_2-scavenging, phenolic/AsA/POD system. In the present study, the increment of total polyphenol (TPC) and flavonoid content (TFC) were depended on degree of water stress (Fig 4c, 4d)). Reddy *et al.* [199] in higher plant reported ameliorate response of TPC and TFC under drought stress.

Total antioxidant activity

Total antioxidant content of leaves had significant ($p < 0.01$) and remarkable increase across all cultivars under all drought stresses in following order: control < LDS < MDS < SDS (Fig. 4e). VA16 had the highest TAC content from LDS to SDS followed by VA11 while at control the highest TAC was observed in VA11 followed by VA16. In contrast, the lowest TAC was recorded in VA6 under all drought stresses.

Total antioxidant activity is the combined results of all enzymatic and non-enzymatic antioxidants activity in natural and/or biotic/abiotic stress. Tolerant plant genotypes usually have a better antioxidant content to protect them from oxidative stress by maintaining high antioxidant enzyme and antioxidant molecule activity and contents under stress conditions. Antioxidants protect the cells from free radicals and therefore have been considered as a method to improve plant defense responses [208]. Water stress can lead to elevation of reactive oxygen species and, therefore, higher amounts of antioxidants is required to compensate stress condition and increase the tolerance [209]. Antioxidant activity has a crucial role in maintaining the balance between the production and scavenging of free radicals [210]. The observed positive correlations among total carotenoid, ascorbic acid, TPC, TFC and TAC (see supplementary Table S1) indicated that the increase in any one of these antioxidant activity was accompanied by an enhancement in each of the five antioxidant activity, presumably as a result of high demand for quenching H_2O_2. It can most likely be inferred that total carotenoid, ascorbic acid, TPC, TFC and TAC correspondingly organize in relation to each other.

This investigation provided an impact of drought stress on the ROS marker, physiological and biochemical parameters in four *A. tricolor* cultivars. The reported results exhibited substantial drought effects on the measured parameters with a significant and differential cultivar responses. Nevertheless, response of ROS marker, physiological and

biochemical parameters was different in respect to cultivars and the degree of drought stress. Responses of VA14 and VA16 to ROS marker, physiological and biochemical parameters assumed that these cultivars are promising with appropriate tolerance to drought stress. Therefore, these two cultivars could be used as tolerant cultivar. Positively significant correlations among ROS marker (MDA, H_2O_2), compatible solutes and non-enzymatic antioxidant (proline, TPC, TFC and TAC) suggested that compatible solutes and non-enzymatic antioxidant played vital role in detoxifying of ROS in *A tricolor* cultivar. Nevertheless, it should be confirmed for a wider range of drought stress as well as over a wider range of environmental conditions. The increased content of ascorbic acid, indicates the crucial role of the ASC–GSH cycle for scavenging ROS in leaves of *A. tricolor*. A thorough investigation should be conducted with the aim of understanding the detail insight into ASC–GSH cycle of *A. tricolor* cultivars under drought stress.

Abstract

Four selected *A. tricolor* cultivars were grown under four irrigation regimes (25%, 50%, 80% and 100% field capacity) to evaluate the mechanisms of growth, physiological and biochemical responses against drought stress in randomized complete block design with three replications. Drought stress led to decrease in total biomass, specific leaf area, RWC, photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, chlorophyll *ab*), soluble protein and increase in MDA, H_2O_2, EL, proline, total carotenoid, ascorbic acid, polyphenols, flavonoids and antioxidant activity. However, responses of these parameters were differential in respect to cultivars and the degree of drought stresses. No significant difference was observed in control and LDS for most of the traits. The cultivars VA14 and VA16 were identified as more tolerant to drought and could be used for further evaluations in future breeding programs and new cultivar release programs. Positively significant correlations among MDA, H_2O_2, compatible solutes and non-enzymatic antioxidant (proline, TPC, TFC and TAC) suggested that compatible solutes and non-enzymatic antioxidant played vital role in detoxifying of ROS in *A tricolor* cultivar. The increased content of ascorbic acid, indicated the crucial role of the ASC–GSH cycle for scavenging ROS in *A. tricolor*.

3.2.2 Catalase, superoxide dismutase and ascorbate-glutathione cycle enzymes confer drought tolerance of *Amaranthus tricolor*

Purpose of the study

Drought stress causes oxidative stress by decreasing stomatal conductivity that confines CO_2 influx in to the leaves. This reduces the leaf internal CO_2, which leads to the formation of ROS such as hydroxyl radicals (OH•) singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), alkoxyl radical (RO) and superoxide radical ($O_2^{\bullet-}$) mainly by enhancing electrons leakage to oxygen molecule [82-85]. In plant cell, mitochondria, chloroplasts and peroxisomes are the main locations of ROS generation [86]. In addition, Environmental stress stimulates xanthine oxidase in peroxisomes, amine oxidase in the apoplast and NADPH oxidases (NOX) in the plasma membrane and produce ROS [87, 88]. Environmental stress induces excess ROS that can injure plant cells by oxidation of cellular components such as proteins, inactivate metabolic enzymes, DNA and lipids [89, 90].

The response of plant defense system to stress varies with the times, duration of contact and stress severity, type of organ or tissue and developmental stage [91, 92]. At a certain level, ROS works as an indicator molecule for activating acclimatory/protection responses through transduction pathways, where H_2O_2 acts as a secondary messenger [93, 94]. However, additional ROS induces harmful effects on plant cells. As a result, defenses against ROS are activated [98] by an array of nonenzymatic antioxidants [metabolites such as ascorbate (AsA), carotenoids, glutathione (GSH) and proline] and antioxidant enzymes [such as guaiacol peroxidases (GPOX), catalase (CAT), superoxide dismutase (SOD) and AsA-GSH cycle enzymes like glutathione reductase (GR) ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR)], work together for detoxification of ROS [87, 88, 96-101]. In glutathione-ascorbate cycle, reduced glutathione is produced from oxidized glutathione through the donated electrons of all nonenzymatic and enzymatic antioxidants [89]. In addition to their damaging effects on cells, ROS can also take part as signaling molecules in many biological processes such as growth, enclosure of stomata, stress signaling and development [90, 102-104]. Recently more attention has been given to understand the antioxidant defense mechanism in plants exposed to drought stress [105-107]. Abiotic stress enhances the production of AsA–GSH and AsA–GSH cycle enzymes activities for cellular protection. Plant water relations play a significant role in the stimulation and/or modulation of antioxidative defense mechanism at drought stress [108-110].

In Bangladesh, *A. tricolor* is very cheap and common leafy vegetable. It grows widely in Southeast Asia, Africa, arid and semiarid regions around the globe. There is no information

on mechanism of water deficit tolerance of *A. tricolor* genotypes in relations to antioxidative defense system in ROS detoxification. In our previous studies [143, 149-151, 160-162, 173] we selected some high yielding potential genotypes rich in antioxidant content. We also found tremendous increment of ascorbic acid under drought [211] and salinity [212] stress and APX [213] with the severity of drought stress in selected genotypes. This result grew many interests to study the role of antioxidant enzymes especially AsA-GSH cycle pathway for enhancing the protection of *A. tricolor* from oxidative stress under drought stress. In this study, we want to elucidate key physiological, enzymatic and non-enzymatic pathways involved in ROS detoxification and tolerance of *A. tricolor* under drought stress.

Materials and methods

Plant materials and experimental conditions

We selected one drought tolerant (VA13) and one moderately drought sensitive (VA15) *Amaranthus tricolor* varieties on the basis of our previous morphological and physiological study (Data not published). These two varieties were grown in pots of a rain shelter open field of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh (AEZ-28, $24^0 23'$ north latitude, $90^0 08'$ east longitudes, 8.4 m.s.l.). The trial area remains covered during rainfall events and otherwise exposes plants to ambient field conditions. Topsoil layer of the experimental station was collected from 30 cm depths for the potting soil. The soil was silty clay with slightly acidic (pH 6.4) and low in organic matter (0.87%), total N (0.09%) and exchangeable K (0.13 c mol kg^{-1}). The soil S content was at par with critical level, while P and Zn contents were above the critical level (Critical levels of P, S, and Zn were 14, 14 and 0.2 mg kg^{-1}, respectively and that of K was 0.2 c mol kg^{-1}). The seeds were sown in plastic pots (22 cm in height and 60 cm length and 40 cm width) maintaining 20 cm apart rows and 5 cm from plant to plant distance. The experiment comprised two factors (drought level and genotype) in a factorial fashion in a randomized complete block design (RCBD) with four replications. Total 36 pots were sown with 18 pots per variety and 12 pots per treatment. Fertilizer was applied to the rate of 92:48:60 kg ha^{-1} $N:P_2O_5:K_2O$ as a split dose. First, in pot soil, at the rate of 46:48:60 kg ha^{-1} $N:P_2O_5:K_2O$ and second, at 10 days after sowing (DAS) at the rate of 46:0:0 kg ha^{-1} $N:P_2O_5:K_2O$. The average day/night temperatures, relative humidity and day length during the experimental period was 25/21 °C, 74%, and 12 h, respectively. Each variety was grouped into four sets and subjected to three drought stress treatments that are, control (Cont., 100% FC); moderate drought stress (MDS, 60% FC); and severe drought stress (SDS, 30% FC). At first, pot soil field capacity was measured by the gravimetric method. Then

the amount of water at field capacity was measured by subtracting the weight of completely dry soil from the weight of soil at field capacity. Pot weight (including pot soil) for each treatment was calculated by weighing of completely dry soil and amount of water required for attaining respective field capacity. Pots were well-irrigated every day up to 10 DAS for dynamic growth and proper establishment of seedlings. Imposition of water stress treatment was started at 25 DAS. Pots were weighed twice a day at 12 h intervals. To achieve the target field capacity of each water condition, the amount of water equaling that lost through transpiration and soil evaporation, percolation and leaching were added. Water stress was imposed up to 55 DAS. The leaves of *A. tricolor* were harvested at 55 DAS. Sampling was completed between 11:00 and 12:00. For quantification of plant parameters, fully emerged top young leaves from control and stressed plants were sampled. All the parameters were measured in four replicates.

Plant growth measurements

At 55 DAS, total biomass and SLA were measured from 5 plants. LI-3100 leaf area meter (LICOR. Inc., Lincoln, NE, USA) was used to determine total leaf area per plant. the samples were oven dried at 70 °C until constant weight achieved. The dry mass of total plant and leaves was taken. For determination of SLA, total plant leaf area was divided by the leaf dry weight.

Determination of chlorophylls and total carotenoid content

Chlorophyll, total carotenoids, betacyanin and betaxanthin content were measured following the procedure described in the previous chapter

Determination of leaf relative water content and electrolyte leakage

Leaf relative water content and electrolyte leakage were measured following the procedure described in the previous chapter

Determination of leaf malondialdehyde, soluble protein, proline and H_2O_2

Malondialdehyde, soluble protein, proline and H_2O_2 concentrations were measured following the procedure described in the previous chapter

Determination of antioxidant.

Leaf samples were prepared for AsA, DHA, GSH and GSSG analyses by homogenizing 1 g leaf material (F. wt.) in 10 ml of cold 5% sulphosalicylic acid [214]. The homogenate was centrifuged at 22000 × g for 15 min at 4 °C, and the supernatant was collected for analyses of ascorbate and glutathione. AsA, DHA and total ascorbate (AsA + DAsA) were measured according to Zhang and Kirkham [214]. DHA was reduced to AsA by adding DTT and total ascorbate was measured. The concentration of DHA was estimated from the difference between total ascorbate and AsA. 0.3 ml aliquots of the supernatant, 0.75 ml of 150 mM phosphate

buffer (pH 7.4) containing 5 mM EDTA, and 0.15 ml of 10 mM DTT were added to determine total ascorbate. To remove excess DTT, 0.15 ml of 0.5% *N*-ethylmaleimide was added after incubation for 10 min at room temperature. Instead of DTT and N-ethylmaleimide 0.3 ml H_2O was added to measure in a similar reaction mixture. After adding 0.6 ml of 10% TCA, 0.6 ml of 44%, orthophosphoric acid, 0.6 ml 4% *α, α′*-dipyridyl 70% ethanol, and 0.3% (w/v) FeCl3 reagents color was developed in both reaction mixtures. After vortex mixing, the mixture was incubated at 40 °C for 40 min and the A_{525} was read. A standard curve in the range 0-100 µg AsA ml^{-1} was prepared. Data were calculated as µmoles per gram dry weight (µmol g^{-1} dw).

GSH and GSSG were assayed according to the methods of Zhang and Kirkham [214]. One ml aliquot of the supernatant was neutralized with 1.5 ml of 0.5 M phosphate buffers (pH 7.5), then 50 µl H_2O was added; this sample was used for the assay of total glutathione (GSH + GSSG). Another 1 ml aliquot of the supernatant was neutralized with 1.5 ml of 0.5 M phosphate buffers, 50 µl of 2-vinylpyridine was added to mask GSH, and the constants of the tube were mixed until an emulsion formed. The tube was then incubated for 60 min at room temperature. This sample was used for the assay of GSSG. GSH was estimated as the difference between total glutathione and GSSG. Glutathione content was measured in a 3 ml reaction mixture containing 0.2 mM NADPH, 100 mM phosphate buffer (pH 7.5), 5 mM EDTA. 0.6 mM DTNB and 3 units of GR. The reaction was started by adding 0.l ml of extract sample obtained as described above. The reaction rate was monitored by measuring the change in absorbance at 412 nm for 1 min. A standard curve was developed based on GSH in the range 0-50 µmol ml^{-1}. Data were calculated as µmoles per gram dry weight (µmol g^{-1} dw).

Determination of antioxidant enzymes activities

1 g of leaf samples were freezed in liquid nitrogen followed by grinding in 10 mL extraction buffer (0.1 M phosphate buffer, pH 7.5, containing 0.5 mM EDTA in case of SOD, GPOX, CAT and 1 mM ascorbic acid in case of APX to prepare the extract. The homogenates were filtered through four layers of cheesecloth and then centrifuged at 4 °C for 20 min at 15000 × g. The supernatant was collected and used for the assays of enzymatic activities. All steps in the preparation of the enzyme extract were carried out at 4 °C.

Total SOD (EC 1.15.1.1) activity was estimated by the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) by the enzyme [215]. 2 mM riboflavin (0.1 mL) was added in 3 mL of reaction mixture (13.33 mM methionine, 75 µM NBT, 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), 50 mM sodium carbonate, 0.1 mL enzyme extract) and placing the tubes under two 15 W fluorescent lamps for 15 min to start the reaction. The absorbance

was recorded at 560 nm, and one unit of enzyme activity was taken as that amount of enzyme, which reduced the absorbance reading to 50% in comparison with tubes lacking enzyme.

Guaiacol peroxidase GPOX (EC 1.11.1.7) activity was measured in terms of increase in absorbance due to the formation of tetra-guaiacol at 470 nm and the enzyme activity was calculated as per extinction coefficient of its oxidation product, tetra-guaiacol $\varepsilon = 26.6\ mM^{-1}\ cm^{-1}$ [216]. 50 mM phosphate buffer (pH 6.1), 16 mM guaiacol, 2 mM H_2O_2 and 0.1 mL enzyme extract were mixed in the reaction mixture. The mixture was diluted with distilled water to make up the final volume of 3.0 mL. Enzyme specific activity is expressed as µmol tetra-guaiacol formed per min per mg protein.

Catalase (EC 1.11.1.6) was assayed by measuring the disappearance of H_2O_2 [217]. 0.5 mL of 75 mM H_2O_2 was added in 1.5 mL of 0.1 M phosphate buffer (pH 7) and 50 µL of diluted enzyme extract in 3 mL reaction mixture. The decrease in absorbance at 240 nm was observed for 1 min and enzyme activity was computed by calculating the amount of H_2O_2 decomposed.

Ascorbate peroxidase (EC 1.11.1.1) was assayed by recording the decrease in optical density due to ascorbic acid at 290 nm [218]. 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA, 0.1 mM H_2O_2, 0.1 mL enzyme and water to make a final volume of 3.0 mL in which 0.1 mL of H_2O_2 was added to initiate the reaction. The decrease in absorbance was measured spectrophotometrically and the activity was expressed by calculating the decrease in ascorbic acid content using a standard curve drawn with identified concentrations of ascorbic acid.

1 ml of 50 mM potassium phosphate buffer (pH 7.0), containing 10% (w/v) polyvinylpyrrolidone (PVP), 0.25% (v/v) Triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM ASA were added in a homogenate of 100 mg (FW) of leaf tissues to measure glutathione reductase (GR, EC 1.6.4.2), dehydroascorbate reductase (DHAR, EC 1.8.5.1), and monodehydroascorbate reductase (MDHAR, EC 1.6.5.4). Murshed *et al.* [219] methods were used to determine GR, DHAR and MDHAR activities. A microplate reader (Synergy Mx, Biotek Instruments Inc., Winooski, VT, USA) were used for determination of all activities and scaled down for semi-high throughput to obtain linear time and protein concentration dependence.

Statistical Analysis

Data were analyzed according to the procedure described in the previous chapter

Results and discussion

Variety, drought stress, and variety × drought stress interactions were significantly different for all the studied traits ($P > 0.01$).

The results of the present investigation suggested that *A. tricolor* is tolerant to drought stress. We select one tolerant and one sensitive *A. tricolor* genotype previously screened for drought stress based on morphological and physiological traits to elucidate key non-enzymatic, physiological, and antioxidant enzymatic defense mechanisms involved. The above defense mechanisms significantly varied in the tolerant and sensitive varieties as are discussed in detail in the following sections.

Response to drought stresses on plant growth, photosynthetic pigment, and relative water content

The major growth parameters, such as total biomass and specific leaf area (SLA); photosynthetic pigment biosynthesis, such as leaf relative water content, chlorophyll *a* and chlorophyll *b* of both varieties reduced significantly under moderate drought stress (MDS) and severe drought stress (SDS) conditions compared to control condition (Fig. 1a-1e). The decline in total biomass, specific leaf areas, chlorophyll *b,* chlorophyll *a* content and RWC of VA15 were much greater compared to VA13 in all the treatments (Fig. 1a-1e). Total biomass, specific leaf area, chlorophyll *a,* chlorophyll *b* content and RWC of VA15 were declined by 28%, 8%, 44%, 71% and 24% under MDS and 59%, 16%, 58%, 56% and 30% under SDS conditions, while total biomass, specific leaf area, chlorophyll *a,* chlorophyll *b* content and RWC of VA13 were declined by 12%, 2%, 18%, 28% and 5% under MDS and 21%, 4%, 8%, 19% and 10% under SDS conditions, respectively compared to control conditions.

Growth is a primary process that affects drought [85]. Total biomass of both varieties of *A. tricolor* significantly declined to MDS and SDS conditions, in comparison with control treatment, indicating that drought stress declined the growth of both varieties. Whereas the tolerant variety showed less decline in total biomass. These results were in full agreement with the results of Sekmen *et al*. [220] who observed that the growth rate of tolerant M-503 cultivar was less affected from drought treatments as compared to the sensitive 84-S cultivar. In our earlier study, we observed decrease in RWC and biomass reduction with the increment of drought stress [213]. Previous studies also have shown that drought stress inhibited growth and RWC in strawberry [97], xerophyte *Capparis* ovata [221] and cotton [220]. It might be accredited to prevent cell elongation and expansion [222, 223], reduction of turgor pressure, changes of energy from growth to biosynthesis of metabolites to preserve turgor pressure of cell, declines in absorption of water that ultimately reduces water content of cell and nitrogen

assimilation [224, 225], reduces the photo-assimilation [226] and metabolites for cell division [189]. In the present investigation, an indicator of leaf thickness SLA had a sharp decline with the increment of drought stress in both varieties under MDS and SDS conditions in comparison with control treatment. Guerfel *et al.* in olive [190] and Sarker and Oba in *Amaranthus* [213] observed a similar trend of decline in SLA.

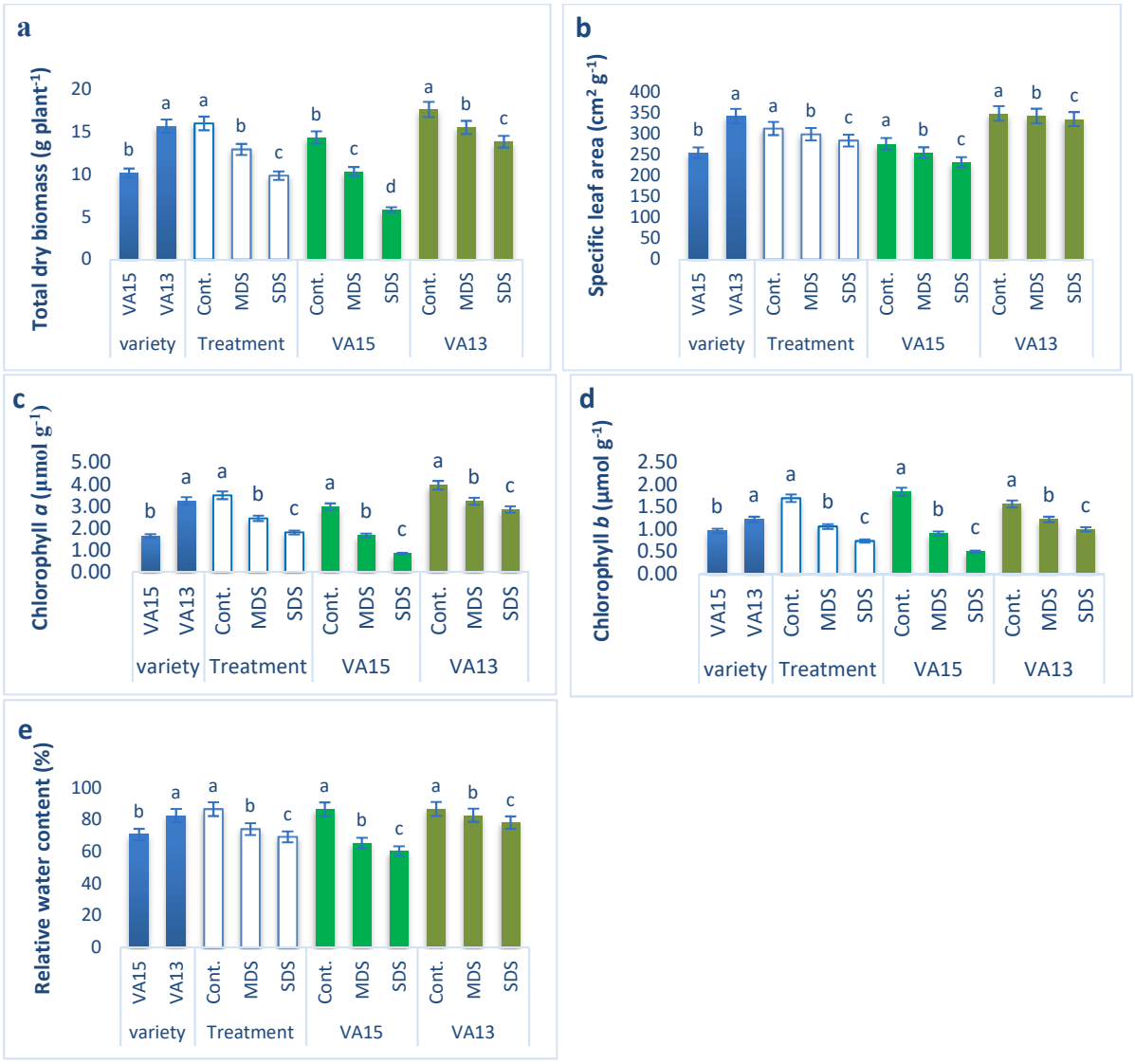

Fig. 1. Effect of drought stress on growth, photosynthetic pigment biosynthesis and leaf relative water content (RWC%) in *A. tricolor.* Cont., control (100% FC); MDS (60% FC), moderate drought stress; SDS (30% FC), severe drought stress; total dry biomass (1a); specific leaf area (1b); chlorophyll *a* (1c); chlorophyll *b* (1d) and leaf relative water content (1e); Values are means of three replicates and different letters are differed significantly by Duncan multiple Range Test ($P < 0.01$).

Growth and SLA reduction in sensitive genotype VA15 were significantly higher than that of tolerant genotype VA13 under both MDS and SDS conditions i.e., VA13 showed better adaptation compared to VA15. Similarly, Zheng *et al*. [227] also found different adaptation in two genotypes of *C. bungee.* In this study, VA13 had more chlorophylls content and less decline in chlorophylls than VA15, suggesting that VA13 was more drought tolerant compared to VA15. Sarker and Oba [213] in *A. tricolor*, Shahbaz *et al*. [228] in wheat and Zhang and

Kirkham [214] in sorghum and sunflower observed a decline in leaf chlorophyll contents under drought stress conditions. Drought stress induces the oxidation of chlorophyll pigment resulting in decrement of chlorophyll pigments [194], chloroplasts disruption or augmented activity of chlorophyllase [195]. *A. tricolor* has betacyanin and betaxanthin that absorb a substantial amount of radiation which ultimately protects chloroplasts from harmful excessive light under stressful condition [198]. In the present study, this might be the reason for lower chlorophyll reduction in both varieties. RWC is convenient attributes for assessing physiological hydration condition of crops and its metabolism and existence. It might be utilized for distinguishing between sensitivity and tolerance in drought-stressed crops [191]. Both *A. tricolor* varieties resulted in a drought-induced reduction of RWC under MDS and SDS conditions, compared to the control treatment, respectively, however, the reduction was more drastic in VA15 compared to VA13. In our previous studies, we also found similar results in *A.* tricolor [213]. Munne-Bosch and Penuelas [97] in strawberry, Ozkur *et al*. [221] in xerophyte *Capparis ovata* and Sekmen *et al*. [220] in cotton observed a similar decline in RWC under drought stress. Drought stress reduces turgor pressure, decreases available water in the soil, hampers roots water absorption, finally results in decrease in RWC of leaves. Under drought stress, VA13 exhibited higher RWC; it might be due to probable osmoregulation approach and greater antioxidants accumulation under drought stress in comparison to VA15 [213]. Turkan *et al*. [229] and Cia *et al*. [230] showed that tolerant varieties have maintained better RWC under drought stress. Thus, VA13 seemed to be more capable to decrease the cellular osmotic pressure and to permit the roots for absorbing adequate water to sustain cell turgor pressure and for taming potentiality against hydration status.

Influence of drought stresses on lipid peroxidation, hydrogen peroxide, and EL%

MDA, H_2O_2 content and EL% augmented progressively with the increment of drought stress in the sensitive variety VA15 under MDS and SDS conditions, whereas the increments of EL% in the tolerant variety VA13 under MDS and SDS conditions were much lower compared to control condition. In contrast, there were no increments of MDA and H_2O_2 content in the tolerant variety VA13 under MDS and SDS conditions compared to control treatment. (Fig. 2a, 2b, 2c). EL% in the tolerant variety VA13 were increased by 103% under MDS and 233% under SDS conditions, while MDA, H_2O_2 content and EL% of sensitive variety VA15 were rapidly increased by 107%, 76%, and 331% under MDS and 173%, 137% and 495% under SDS conditions, compared to control conditions, respectively. Drought stresses intensify the manufacture of ROS like alkoxy radicals, $O_2^{\bullet-}$, singlet oxygen, H_2O_2, OH• etc. which ultimately create oxidative stress in cell [213, 231]. Primary stimulation of O_2 by xanthine oxidase, $O_2^{\bullet-}$

dismutation, electron reduction at higher O_2 level are the main mechanisms of ROS generation in plants [232]. ROS causes oxidative stress through damage DNA, lipids and proteins, restricting the normal cell functions. Drought stress aggravates ROS production in chloroplasts, mitochondria and peroxisomes [86,189].

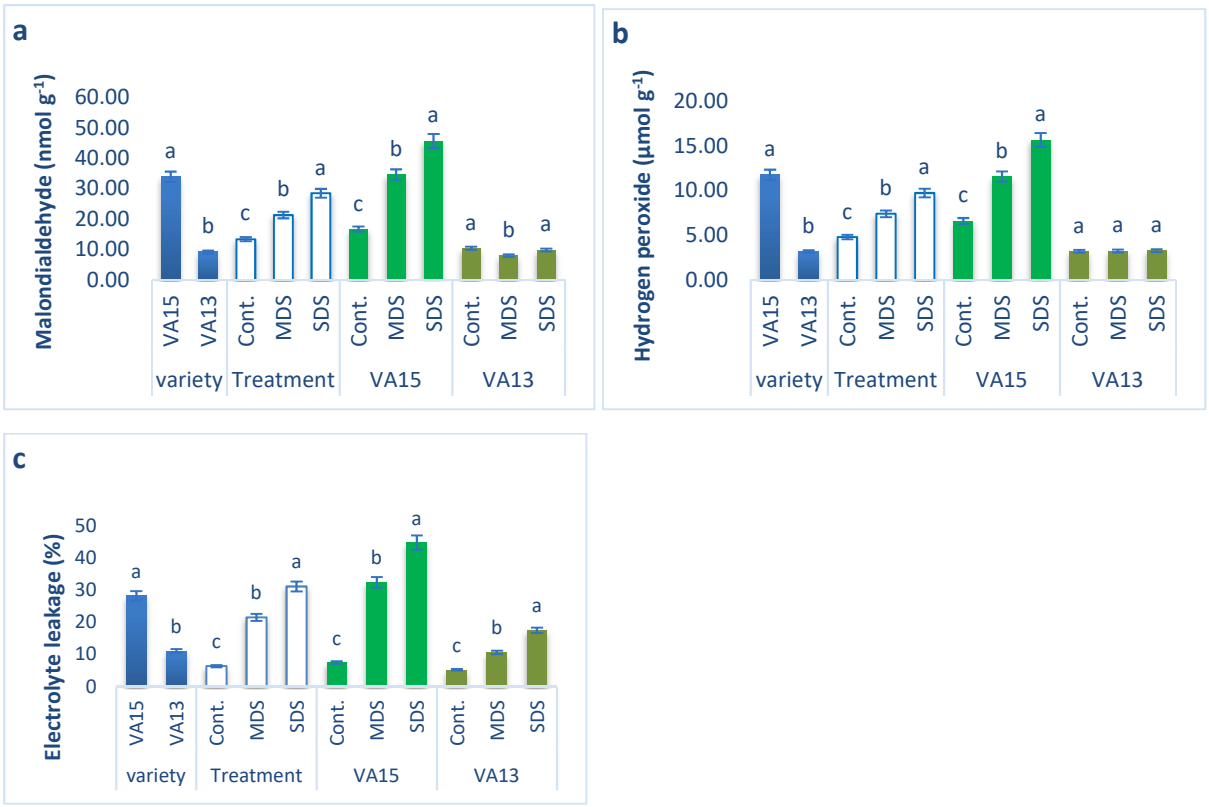

Fig. 2. Influence of drought stress on malondialdehyde content (MDA, 2a); hydrogen peroxide (H_2O_2, 2b), electrolyte leakage (EL%, 2c) in *A. tricolor*. Cont., control (100% FC); MDS (60% FC), moderate drought stress; SDS (30% FC), severe drought stress; Values are means of three replicates and different letters are differed significantly by Duncan multiple Range Test ($P < 0.01$).

In our study, we found a substantial production of H_2O_2, lipid peroxidation and increase in EL in the sensitive variety (VA15) of *A. tricolor* under drought stress. EL leakage was much greater in the sensitive variety (VA15) as compared to the tolerant variety (VA13). These results agreed with the results of our previous study in amaranth [213], Christou *et al*. [200] in strawberry and Chakraborty *et al*. [232] in groundnut. Our results clearly demonstrated that at similar drought stress, the sensitive *A. tricolor* accumulated more ROS compared to the tolerant variety. Hence the tolerant variety maintained the ROS to a relatively lower level than sensitive variety. In the present investigation, extreme accumulation of H_2O_2 at MDS and SDS in the sensitive variety might be due to acceleration of the Haber-Weiss reaction that causing formation of hydroxyl radical (•OH), hence, resulting in more MDA production and damage of cell membrane [89]. At stressful conditions, it is crucial to maintaining a balance between ROS assembly and detoxification [201]. In our study, drought-stressed conditions remarkably augmented non-enzymatic and enzymatic antioxidants by defensive techniques from MDS to

SDS to lessen EL, H_2O_2 and MDA accumulation. The tolerant cultivars VA13 had very low H_2O_2 and MDA content. The tolerant cultivar improved the stressful condition by several protection ways, such as non-enzymatic antioxidant, antioxidant enzymes and AsA-GSH cycle which inhibited drought stress impact by protection of ROS generation. Under water stress, electrolyte leakage is considered to be a symbol of damage and descent [233]. In the present investigation, drought stress progressively enhanced electrolyte leakage. Hence, electrolyte leakage might be used to distinguish stress-susceptible and tolerant cultivars. Abiotic stress tolerance is associated with lower electrolyte leakage. The Severity of drought-induced progressive increment in MDA and H_2O_2 that enhanced the damage of cell membrane in the sensitive variety and demonstrated by a sharp increase in EL. Tolerant genotype VA13 showed lower electrolyte leakage compared to sensitive genotype.

Effect of drought stresses on proline, total carotenoids, ascorbate, glutathione content

Proline content was augmented significantly with the increment of drought stress in VA15 under MDS and SDS conditions, while total carotenoids reduced from control to MDS and which was statistically similar at MDS and SDS conditions. Proline increments in VA13 under MDS and SDS conditions were comparatively much lower than in VA15 compared to control condition, while total carotenoids increment in VA13 under MDS and SDS conditions were comparatively higher than in VA15 compared to control condition (Fig. 3a, 3b). Proline of VA15 was increased by 248% under MDS and 566% under SDS conditions, respectively when compared with control treatment. In contrast, proline and total carotenoids of VA13 were increased by 72% and 20% under MDS and 176% and 55% under SDS conditions, respectively in comparison with control treatment. Ascorbate, ascorbate/total ascorbate redox status, glutathione and glutathione/total glutathione redox status remarkably augmented with the increment of drought stress in VA13 under MDS and SDS conditions, while ascorbate, ascorbate redox, glutathione and glutathione redox increments in VA15 under MDS and SDS conditions were much lower than in VA13 compared to control condition, respectively (Fig. 3c, 3d, 3e, 3f). Ascorbate, ascorbate redox, glutathione and glutathione redox of VA13 were increased by 158% 15%, 45% and 9% under MDS and 286% 37% 98% and 29% under SDS conditions, whereas ascorbate, ascorbate redox, glutathione and glutathione redox of VA15 were increased by 11% 19% 16% and 5% under MDS and 10% 30% 21% and 9% under SDS conditions, respectively compared to control conditions. Proline content of both the varieties was significantly increased under MDS and SDS conditions, whereas the increment was greater in the sensitive variety VA15 compared to tolerant variety VA13.

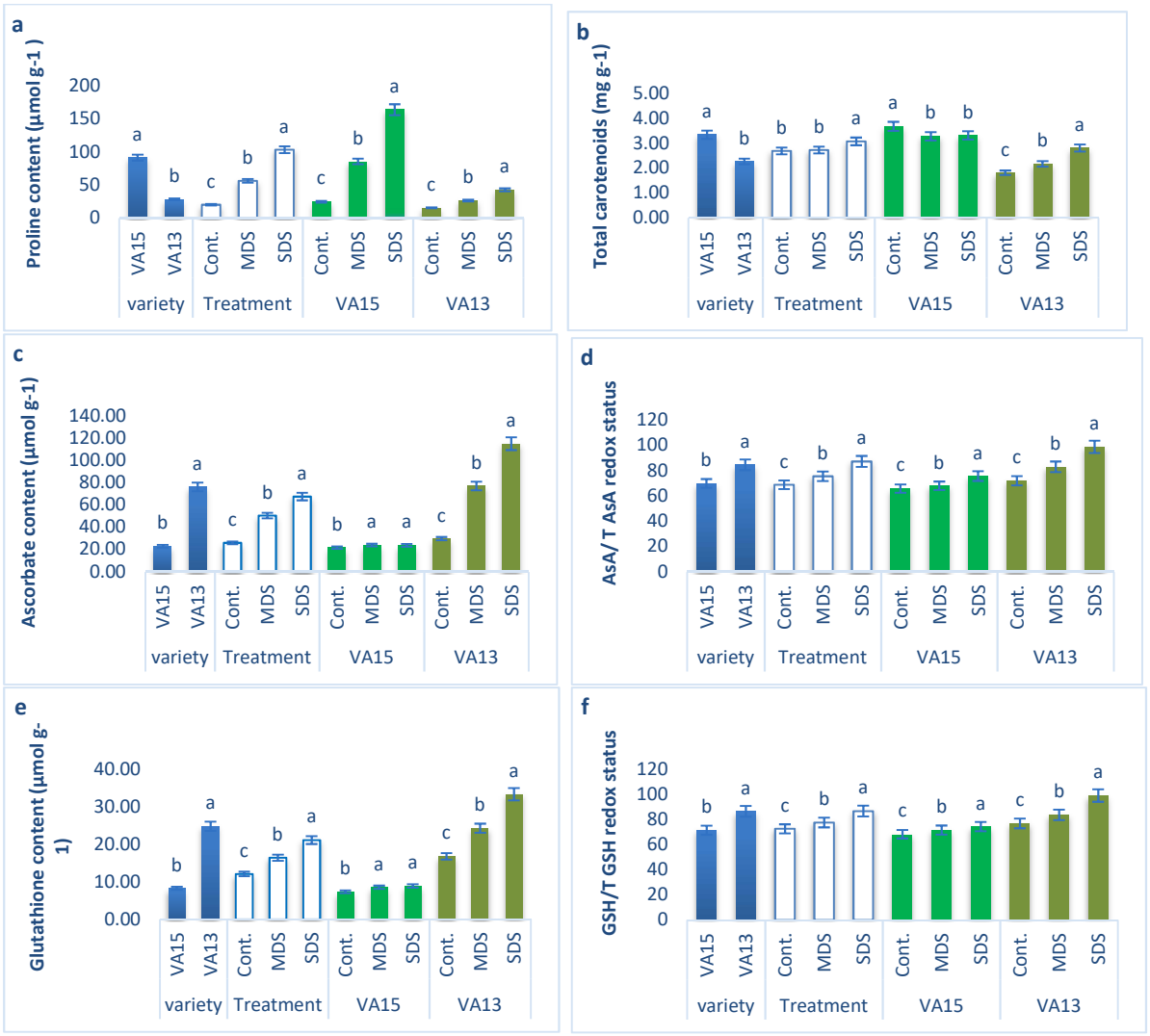

Fig. 3. Effect of drought stress on proline content (3a); total carotenoid (3b); ascorbate content (3c); ascorbate/total ascorbate% (3d); glutathione content (GSH) (3e); glutathione/total glutathione% (3f) in *A. tricolor.* Cont., control (100% FC); MDS (60% FC), moderate drought stress; SDS (30% FC), severe drought stress; Values are means of three replicates and different letters are differed significantly by Duncan multiple Range Test ($P < 0.01$).

It is evident from the results that proline had no significant role in the mechanisms of drought stress tolerance in *A. tricolor*, as a functional osmolyte and antioxidant for adjustment of osmotic stress and ROS detoxification in *A. tricolor* as it accumulates to higher levels in the drought-sensitive variety. Nayyar and Walia [234] and Tatar and Gevrek [235] in wheat, Zheng *et al*. [227] *Catalpa bungee* observed proline increment under drought stress. Carotenoids are capable to scavenge lipid peroxy-radicals and singlet oxygen and inhibit superoxide generation and lipid peroxidation under drought stress [189]. Total carotenoids are lipophilic antioxidants that are capable to purify different types of ROS [203]. In plants, total carotenoid usually absorbs light at 400 and 550 nm and transfer the apprehended energy to the chlorophyll [236]. Carotenoids can act as an antioxidant that inhibits oxidative damage by scavenging $^1O_2$, quenching triplet sensitizer (3Chl*), exciting chlorophyll (Chl*) and protecting the photosynthetic apparatus. Ascorbate (AsA) is one of the powerful antioxidants [205]. AsA and

αtocopherols predominately quench O_2 straightly or by enzymes catalysis. It permits non-enzymatic and enzymatic antioxidative ROS detoxification. AsA scavenges OH, SOR and $^1O_2$ directly and reduces H_2O_2 to water through ascorbate peroxidase reaction [206]. Antioxidant ascorbate and total carotenoid had a vital role in counterbalancing oxidative stress and manipulating homeostasis of ROS in plants [237]. Our results showed that the total carotenoid level was increased in VA13, while the decrement of this compound was observed in VA15. In the tolerant variety VA13, had a remarkable rise in ascorbate-glutathione content and ascorbate-glutathione redox status, while the sensitive variety VA15 exhibited negligible increment of ascorbate-glutathione content and ascorbate-glutathione redox status. For instance, drought and salt stress increased the activity of ascorbate-glutathione content and ascorbate-glutathione redox status in pea [238], wheat [239], sorghum and sunflower [214], *Catalpa bungee* [227], strawberry [97] and groundnut [232], particularly for tolerant lines under water deprivation condition. The AsA-GSH content, AsA-GSH redox status specifies the essential part of the AsA–GSH cycle for detoxification of ROS in the tolerant *A. tricolor.* Similarly, Hernandez *et al.* [238] reported that salinity stress accumulated higher transcripts of the AsA–GSH cycle in the tolerant variety compared to the sensitive variety.

Effect of drought stresses on antioxidant enzymes activities

CAT and SOD activities progressively augmented with the increment of drought stress under MDS and SDS conditions in comparison with control treatment in both varieties, however, the increments of SOD and CAT activities in VA13 were higher compared to VA15 at all drought stress levels (Fig. 4a, 4c). CAT and SOD activities of VA13 were increased by 28% and 53% under MDS and 70% and 105% under SDS conditions, whereas CAT and SOD activities of VA15 were increased by 48% and 64% under MDS and 76% and 94% under SDS conditions,respectively compared to control treatment. The GPOX activity significantly and remarkably augmented with the increment of drought stress under MDS and SDS conditions in comparison with control treatment in both varieties, while VA15 exhibited the highest increments compared to VA13 at all drought stress treatment (Fig. 4b). The GPOX activity of VA13 was increased by 9% and 23% at MDS and SDS conditions, whereas GPOX activity of VA15 was increased by 18% and 29% at MDS and SDS conditions, respectively in comparison with control treatment.

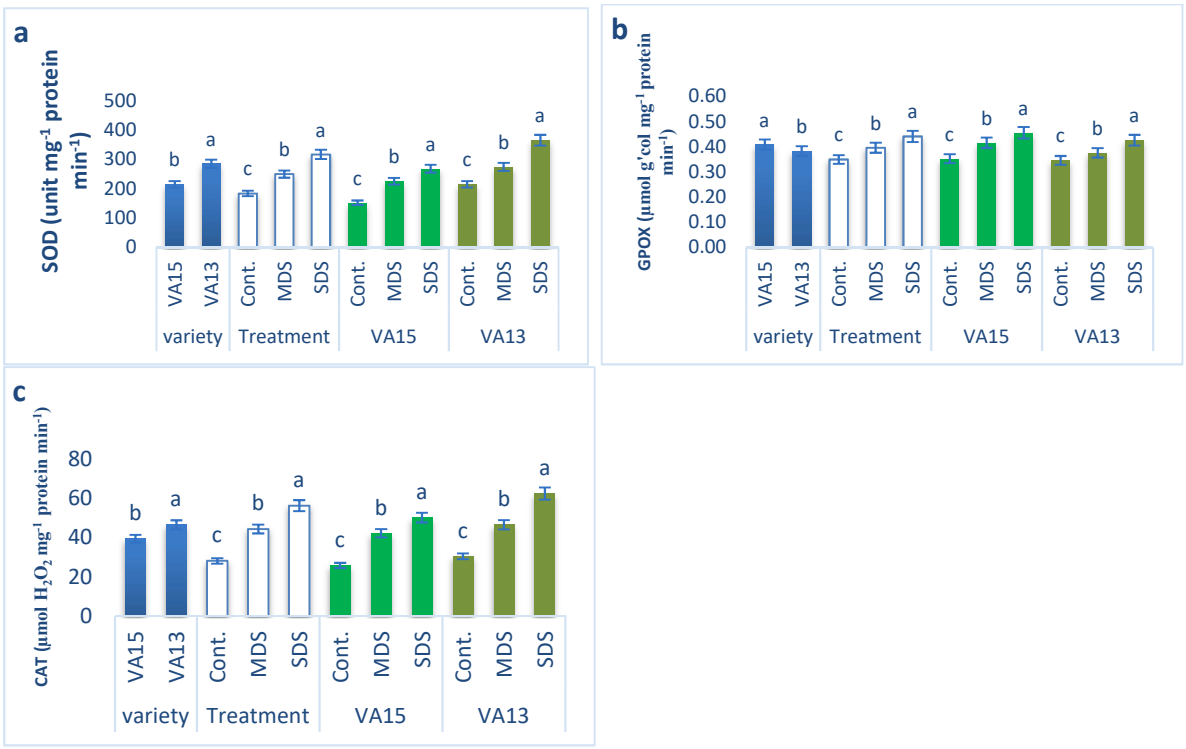

Fig. 4. Response of 4a, super oxide dismutase (SOD) (unit mg^{-1} protein min^{-1}); 4b, guaiacol peroxidase (GPOX) (µmol g'col mg^{-1} protein min^{-1}); 4c, catalase (CAT) (µmol H_2O_2 mg^{-1} protein min^{-1}) enzymes on drought stress in *A. tricolor.* Cont., control (100% FC); MDS (60% FC), moderate drought stress; SDS (30% FC), severe drought stress; Values are means of three replicates and different letters are differed significantly by Duncan multiple Range Test ($P < 0.01$).

Effect of drought stresses on AsA-GSH cycle enzymes activities

MDHAR, DHAR, APX and GR activity progressively augmented with the increment of drought stress under MDS and SDS conditions in comparison with control treatment in the tolerant genotype VA13, while the increments of those enzymes' activities were much lower in the sensitive genotype VA15 compared to tolerant genotype VA13 at all drought stress levels (Fig. 5a, 5b, 5c, and 5d). MDHAR, DHAR, APX and GR activity of VA13 were augmented by 125% 125%, 122% and 124% under MDS and 379%, 375%, 371% and 375% under SDS conditions, whereas MDHAR, DHAR, APX and GR activity of VA15 were augmented by 45% 40%, 37% and 2% under MDS and 70%, 63%, 64% & 20% under SDS conditions, respectively compared to control condition.

Drought stress generated superoxide from photosynthetic and respiratory electron leakage in chloroplast. Superoxide dismutase (SOD) enzyme dismutated superoxide into H_2O_2. H_2O_2 was decomposed by different peroxidases such as ascorbate peroxidase (APX), glutathione peroxidase (GPX) and phenol peroxidase [86] into the water by using various reducing agents. In contrast, catalase (CAT) mostly decomposed photorespiration mediated H_2O_2 in the peroxisome [91]. In this study, we found that drought stress induced CAT and SOD

activities in both varieties whereas, CAT and SOD activities were much greater in the tolerant variety VA13 compared to sensitive variety VA15, suggesting role of CAT and SOD in drought tolerance in *A tricolor* by detoxification of H_2O_2 and activating dismutation reaction to alter SOR to hydrogen peroxide, respectively. These results agreed to results of Ben Amor et al. in halophyte *Cakile maritima* [240] where they interrelated in increased SOD activity with plant salt tolerance. Khanna-Chopra and Selote [239] in wheat, Ozkur *et al*. [221] in *Capparis ovata*, Zhang and Kirkham [214] in sorghum and sunflower and Chakraborty *et al*. [232] in groundnut observed enhanced activities of SOD, POX and CAT under drought and salt stress. Sekmen *et al*. [220] found that the sensitive genotype 84-S associated with decreased activities of catalase (CAT) and peroxidase (POX) to combined stress while the tolerant genotype M-503 was associated with higher activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX) and induced CAT and POX at combined drought and heat stress. In contrast, GPOX had significant and remarkable increasing activity under drought stress, in both varieties, while sensitive variety, VA15 exhibited the highest increase compared to VA13 at all drought stress treatments. Drought stress accelerated higher GPOX increase in the sensitive variety compared to the tolerant variety; it is clearly evident that GPOX had a significant role in enhancing APX activity in the sensitive variety at greater H_2O_2 concentration.

There was a slight and negligible increase in GR, MDHAR, APX and DHAR activity in sensitive variety VA15 under drought stress, while tolerant variety VA13 exhibited the greatest dramatic increase in GR, MDHAR, APX and DHAR activity under drought stress. Hernandez *et al.* [238] in pea found increased activities of GR, MDHAR, APX and DHAR while Chakraborty *et al*. [232] in groundnut showed APX increment under salt stress. Similarly, Khanna-Chopra and Selote [239] and Ozkur *et al*. [221] found that increased activities of APX and GR were associated with drought stress. It indicated that at lower H_2O_2 load, GR, MDHAR, APX and DHAR performed as a main ROS scavenging enzyme in *A. tricolor* under drought stress that may have related to satisfactory regulation of H_2O_2 in the tolerant variety VA13. Increase in AsA-GSH content, reduced AsA-GSH redox status accompanied by AsA–GSH cycle enzymes such as GR, MDHAR, APX and DHAR, clearly evident that AsA–GSH cycle played a crucial role for scavenging ROS in the tolerant variety of *A. tricolor.* Abogadallah *et al*. [241] reported APX-GR as the main H_2O_2 detoxifier at low H_2O_2 load and performed as a satisfactory controller for ROS balancing in barnyard grass under salt stress.

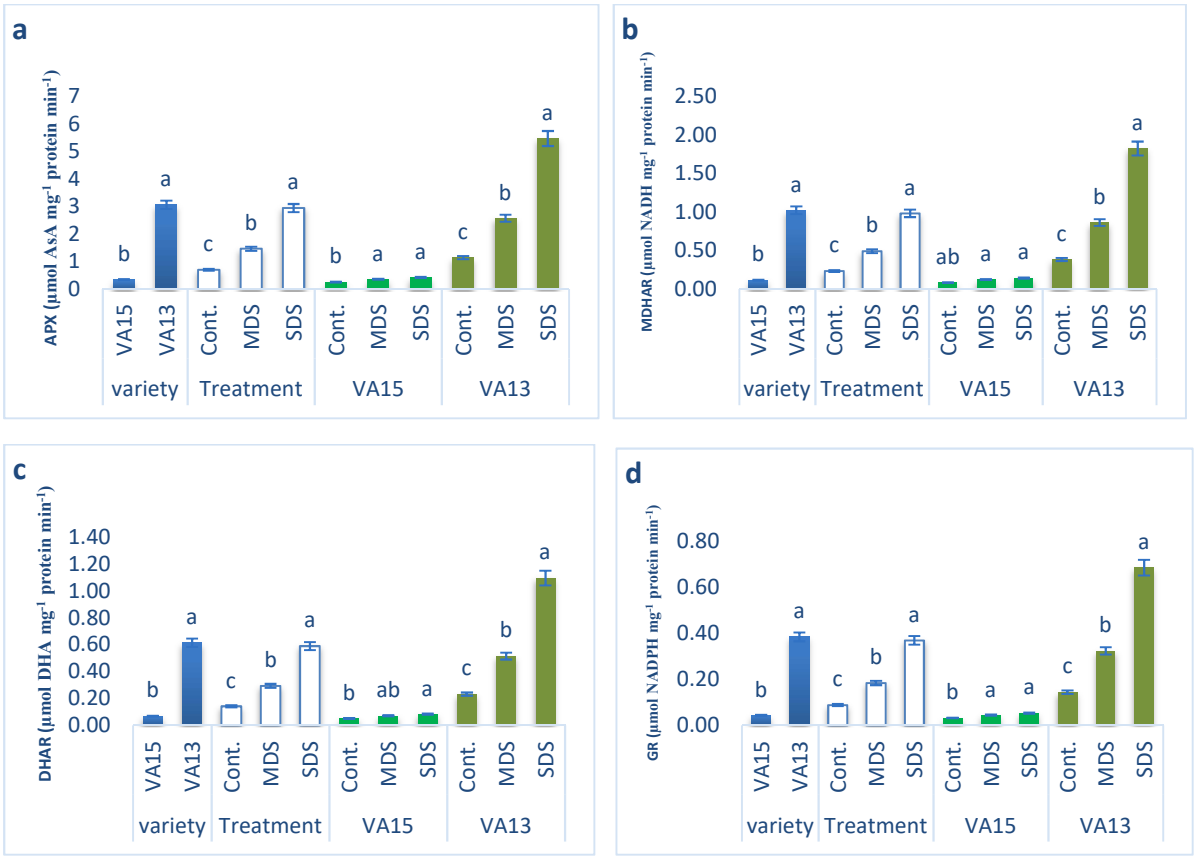

Fig. 5. Response of ascorbate-glutathione cycle enzymes on drought stress in *A. tricolor.* Cont., control (100% FC); MDS (60% FC), moderate drought stress; SDS (30% FC), severe drought stress; [9a, ascorbate peroxidase (APX) (µmol AsA mg^{-1} protein min^{-1}); 5b, monodehydroascorbate reductase (MDHAR) (µmol NADH mg^{-1} protein min^{-1}); 5c, dehydroascorbate reductase (DHAR) (µmol DHA mg^{-1} protein min^{-1}); 5d, glutathione reductase (GR) (µmol NADPH mg^{-1} protein min^{-1}]; Values are means of three replicates and different letters are differed significantly by Duncan multiple Range Test ($P < 0.01$).

The present study concluded that drought stress exhibited differential responses to tolerant and sensitive *A. tricolor* genotypes in terms of growth, physiological, enzymatic and non-enzymatic ROS detoxification pathways involved in the tolerance of *A. tricolor*. Better growth, photosynthetic pigments, RWC, and lower ROS concentration and EL in the tolerant genotype can be recognized to better antioxidative enzymatic protection and cellular antioxidant pool, such as AsA-GSH content, AsA-GSH redox. The present investigation revealed that *A. tricolor* genotype doesn't certainly require concurrent initiation of all antioxidant enzymes for drought tolerance. Only SOD, CAT and AsA-GSH cycle enzymes play a vital role in major ROS detoxification in the tolerant amaranth genotype. Increase in CAT, AsA-GSH content, SOD, AsA-GSH redox and AsA-GSH cycle enzymes activities, clearly evident that AsA–GSH cycle, SOD and CAT play a crucial role in tolerance of *A. tricolor.*

Abstract

The study was performed to explore physiological, non-enzymatic and enzymatic detoxification pathways of reactive oxygen species (ROS) in tolerance of *A. tricolor* under

drought stress. The tolerant genotype VA13 exhibited lower reduction in growth, photosynthetic pigments, relative water content (RWC) and negligible increment in electrolyte leakage (EL), lower increment in proline, guaiacol peroxidase (GPOX) activity compared to sensitive genotype VA15. This genotype also had higher catalase (CAT), superoxide dismutase (SOD), remarkable and dramatic increment in ascorbate-glutathione content, ascorbate-glutathione redox and ascorbate-glutathione cycle enzymes activity compared to sensitive genotype VA15. The negligible increment of ascorbate-glutathione content, ascorbate-glutathione redox and ascorbate-glutathione cycle enzymes activities and dramatic increment in malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and EL were observed in the sensitive genotype VA15. SOD contributed superoxide radical dismutation and CAT contributed H_2O_2 detoxification in both sensitive and tolerant varieties, however, these had a great contribution in the tolerant variety. Conversely, proline and GPOX accumulation were higher in the sensitive variety compared to the tolerant variety. Increase in ascorbate-glutathione cycle enzymes activities, CAT, ascorbate-glutathione content, SOD, and ascorbate-glutathione redox clearly evident that CAT, ascorbate-glutathione cycle and SOD played a significant activity in ROS detoxification of tolerant *A. tricolor* variety.

3.3 Biochemistry and Food Aspect on Salinity Stress of Vegetable Amaranth

A. tricolor is a well-adapted leafy vegetable to different biotic and abiotic stresses and has multipurpose uses. There are few reports related to the effect of salinity stress on leaf pigments, vitamins, phenolic acids, flavonoids and antioxidant capacity in different crops including leafy vegetables. Salt stress elevates protein, ascorbic acid, phenolics, flavonoids and antioxidant activity and reduced fat, carbohydrate, sugar, and chlorophyll pigments in *Cichorium spinosum* [117]. Alam *et al*. [118] observed different levels of salinity treatment resulted in 8–35% increase in TPC; about 35% increase in TFC; and 18–35% increase in FRAP activity in purslane. Lim *et al*. [119] reported that buckwheat treated with 10, 50, 100, and 200 mM NaCl concentrations result in an increase of phenolic compounds and carotenoids in the sprouts compared to the control (0 mM). The buckwheat sprouts treated with 10, 50, and 100 mM NaCl after 7 d of cultivation were 57%, 121%, and 153%, higher phenolic content than that of the control condition, respectively. In plants, polyphenol synthesis and accumulation are mostly stimulated in response to salinity [242].

3.3.1 Salinity stress accelerates nutrients, dietary fiber, minerals, phytochemicals and antioxidant activity in *Amaranthus tricolor* leaves

Purpose of the study

Salinity is one of the major abiotic stressors which limits crop production and poses a serious threat to global food security. Approximately, 20% percent of the arable land and 50% of total irrigated land have varying levels of salinity [111]. Salinity stress induces a multitude of adverse effects on plants including morphological, physiological, biochemical, and molecular changes. It affects plant growth and development by creating osmotic stress, causing specific ions (Na^+ and Cl^-) toxicity, stomatal closure, and reducing the rate of photosynthesis [112]. All these physiological changes in plant under salinity aggravate overproduction of reactive oxygen species (ROS) that interferes normal cellular metabolism and results in oxidative damage by oxidizing proteins, lipids and DNA and other cellular macromolecules [88]. To counterbalance the osmotic stress, plants show variable adaptation processes such as enclosure of stomata, metabolic adjustment, toxic ion homeostasis, and osmotic adjustment [112]. Plants have an excellent network of ROS detoxification system including, either non-enzymatic through protein, carbohydrate, ascorbic acid (AsA), beta-carotene and carotenoids, phenolic compounds and flavonoids or through enzymatic antioxidants, such as superoxide dismutase (SOD), peroxidase (GPOX), catalase (CAT), and AsA peroxidase (APX) [88]. Salinity

tolerance mechanisms in plants are remarkably varied among the species and even within different accessions of a species.

Amaranth is a salt tolerant plant [116]. Salinity stress enhances the contents of natural antioxidants in plants [117-119]. Therefore, salt-stressed plants could economically be the potential sources of antioxidants in human lifestyle. The natural antioxidants in diet play an important role in human health as they are involved in defense against several diseases such as cancer, atherosclerosis, arthritis, cataracts, emphysema, retinopathy, neuro-degenerative and cardiovascular diseases [8. 48, 50]. *A. tricolor* is a well acclimatized leafy popular vegetable to different biotic and abiotic stresses [70]. Various factors such as biological, environmental, biochemical, physiological, ecological and evolutionary processes, and salinity are involved in the quantitative and qualitative improvement of natural antioxidants in this vegetable crop [72]. Salt stress elevated protein, ascorbic acid, phenolics, flavonoids and antioxidant activity and reduced the fat, carbohydrate, sugar, and chlorophyll pigments in *Cichorium spinosum* [117]. Alam *et al*. [118] observed that in purslane, different doses of salt concentrations increased total polyphenol content (TPC); total flavonoid content (TFC); and FRAP activity by 8–35%, 35%, and 18–35%, respectively. Similarly, in buckwheat sprouts, salinity stress remarkably increased phenolic compounds and carotenoids compared to non-saline condition [119]. *A. tricolor* is a popular leafy vegetable in many tropical and subtropical countries. However, no information is available on response of proximate, minerals, vitamins, phenolics, flavonoids and antioxidant activity in the leaves of *A. tricolor* accessions to varying leaves of salinity. In a series of earlier studies [143, 149-151, 160-162, 173], we identified some antioxidant enriched and high yield potential accessions of *A. tricolor*. The central hypothesis of this study was that salinity stress may enhance nutritional contents and antioxidant activities in the leaves of *A. tricolor*. To test this hypothesis, we investigated the response of proximate, minerals, vitamins, phenolics, flavonoids and antioxidant activity in some selected *A. tricolor* accessions to varying levels of salinity stress.

Materials and methods

Experimental site, Plant materials and experimental conditions

We selected three antioxidants enriched high yield potential accessions (Accession VA3, VA12 and VA14) from 102 accessions of Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University, based on our earlier studies [143, 149-151, 160-162, 173]. These accessions were grown in pots of the rain shelter open field of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh (AEZ-28, $24^{0}23^{\prime}$

north latitude, $90^0 08'$ east longitude, 8.4 m.s.l.). The seeds were sown in plastic pots (15 cm in height and 40 cm length and 30 cm width). The experiment comprised a factorial design of salinity treatment and varieties in a randomized complete block design (RCBD) with three replications. Fertilizer was applied at the rate of 92:48:60 kg ha^{-1} N: P_2O_5:K_2O as a split dose. First, in pot soil, at the rate of 46:48:60 kg ha^{-1} N: P_2O_5:K_2O and second, in 7 days after sowing (DAS) at the rate of 46:0:0 kg ha^{-1} N: P_2O_5:K_2O. Each variety was grouped into three sets and subjected to three salinity stress treatments that are, 100 mM NaCl, 50 mM NaCl, and control or no saline water (NS). Pots were well irrigated by fresh water every day up to 10 DAS of seeds for proper establishment and vigorous growth of seedlings. Imposition of salinity stress treatment was started at 11 DAS and continued up to 40 DAS (edible stage). Saline water (100 mM NaCl and 50 mM NaCl) and fresh water were applied to respective pots once a day. At 40 DAS the leaves of *Amaranthus tricolor* were harvested. All the parameters were measured in six samples.

Chemicals and reagents used

Solvent: methanol and acetone. Reagents: ascorbic acid, gallic acid, rutin, methanol, DPPH (2, 2-diphenyl1-picrylhydrazyl), $ABTS^+$, trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid), aluminum chloride hexahydrate, sodium carbonate, potassium acetate, Folin-Ciocalteu reagent, H_2SO_4, NaOH, HNO_3, $HClO_4$, lanthanum, Caesium chloride, dithiothreitol (DTT) and potassium persulfate. All solvents and reagents used in this study were high purity laboratory products obtained from Kanto Chemical Co. Inc. (Tokyo, Japan) and Merck (Germany).

Estimation of proximate composition, mineral content

Proximate composition and mineral content were measured following the procedure described in the previous chapter

Determination of beta-carotene and ascorbic acid

beta-carotene and ascorbic acid content were measured following the procedure described in the previous chapter

Extraction of samples for TPC, TFC and TAC

Samples were extracted following the procedure described in the previous chapter

Estimation of beta-carotene, TPC, TFC and TAC

Beta-carotene, TPC, TFC and TAC were measured following the procedure described in the previous chapter

Statistical Analysis

Data were analyzed following the methods of previous chapter

Results and discussion

Amaranth was considered as the inexpensive leafy vegetables and its cultivation was also limited to Africa, South-East Asia and South America. Recently, amaranth spread over worldwide and its production and consumption have been remarkably increased due to the presence of excellent natural antioxidants such as minerals, antioxidant leaf pigments, carotenoids, vitamins, phenolics and flavonoids. These natural antioxidants have proven health benefits as they detoxify ROS in the human body and involve in defense against several diseases such as cancer, atherosclerosis, arthritis, cataracts, emphysema, retinopathy, neuro-degenerative and cardiovascular diseases [8. 48, 50]. Amaranthus species have higher mineral concentrations than commonly consumed leafy vegetables, such as spinach, lettuce and kale [178]. In *A. tricolor*, iron and zinc content is higher than that of the leaves of cassava [176] and beach pea [177]. The U.S. Department of Agriculture's National Nutrient Database for Standard Reference [243] lists a serving size of spinach as 30 g fresh weight FW (1 cup). As *Amaranthus* has higher mineral concentrations than spinach so, a serving size of leaves of 30 g FW is enough for nutritional sufficiency. In general, leafy vegetables are susceptible salt stress but amaranth is salt tolerant plant [116]. This study comprehensively evaluates the effects of varying levels of salinity stress on contents of nutrients, minerals, dietary fiber, phytochemicals and antioxidant activities of *A. tricolor* accessions. Our results for the first time demonstrated that soil salinity stress up to certain level significantly augment almost all these biochemical parameters in leaves of *A. tricolor*. However, the responses of these parameters to salinity varied among the accessions of *A. tricolor*. Altered proteomes, enhanced vitamins and glycine betaine contents in salinity stressed Amaranthus have previously been reported [116, 244, 245].

Effect of salinity on proximate composition in A. tricolor leaves

The proximate compositions of *A. tricolor* leaves were significantly varied by accessions, salinity levels and accession × salinity stress interactions (Table 1). Among the tested accessions, VA14 had the highest protein (7.25 g 100 g^{-1}), ash content (5.78 g 100 g^{-1}) energy (54.52 Kcal 100 g^{-1}) and the lowest moisture content (81.56 g 100 g^{-1}). However, accession VA12 gave the highest contents of dietary fiber (8.28 g 100 g^{-1}) and carbohydrates (7.06 g 100 g^{-1}). The highest fat content (0.36 g 100 g^{-1}) was recorded in accession VA3. The accession, VA14 had 187%, 50%, and 44% higher protein, ash, and energy contents, respectively compared to the accession VA3. Accession VA12 had 10% and 25% higher carbohydrates and energy, respectively than accession VA3. (Fig. 1). The contents of protein, ash, energy and dietary fiber in *A. tricolor* leaves increased by salinity stress in a level-dependent manner (Fig.

2). The increment of protein, ash, energy and dietary fiber contents in *A. tricolor* by moderate salinity stress (MSS) and severe salinity stress (SSS) were 17, 5, 4 and 15% and 30, 12, 5 and 29%, respectively over no salinity (NS) or control condition. Among salinity stress, NS or control treatment exhibited the highest moisture and fat content, however, moisture and fat contents were the lowest at the SSS conditions. A significant reduction in moisture and fat contents was observed with the increment of salinity stress (control or NS > MSS > SSS). Contents of protein, ash, energy and dietary fiber in plants at SSS conditions were the highest among the salinity stress treatment. The lowest values of these plant parameters were recorded in the control or NS. The highest carbohydrates content (6.21 g 100 g^{-1}) was found in plants grown under SSS, whereas the lowest values (6.15 and 6.17 g 100 g^{-1}) of this parameter were found in NS and MSS treatments, respectively.

Table 1. Salinity effect on proximate composition (per 100 g fresh weight) in three selected *A. tricolor* accessions

Treatment	Moisture (g)	Protein (g)	Fat (g)	Ash (g)	Carbohydrates (g)	Energy (Kcal)	Dietary fiber (g)
Accession × Salinity stress (SS)							
VA3 × NS	88.16 ± 2.13a	2.15 ± 0.04i	0.43 ± 0.01a	3.53 ± 0.05f	5.73 ± 0.02e	33.60 ± 1.12i	6.45 ± 0.07h
VA3 × MSS	87.23 ± 2.08b	2.27 ± 0.04h	0.38 ± 0.02b	3.86 ± 0.06e	6.27 ± 0.05d	35.59 ± 1.20h	7.22 ± 0.09f
VA3 × SSS	85.29 ± 1.56d	3.15 ± 0.02g	0.27 ± 0.03g	4.11 ± 0.05d	7.17 ± 0.04a	41.77 ± 0.89g	8.11 ± 0.08d
VA12 × NS	86.22 ± 2.07c	3.74 ± 0.03f	0.35 ± 0.01c	2.68 ± 0.06h	7.01 ± 0.07c	44.57 ± 1.24f	7.22 ± 0.06f
VA12 × MSS	85.12 ± 1.67d	4.25 ± 0.04e	0.31 ± 0.02e	3.24 ± 0.07g	7.07 ± 0.06b	46.72 ± 1.32e	8.37 ± 0.08c
VA12 × SSS	84.23 ± 1.59e	4.55 ± 0.07d	0.27 ± 0.04g	3.86 ± 0.04e	7.09 ± 0.07b	47.72 ±1.46d	9.24 ± 0.05a
VA14 × NS	82.17 ± 1.54f	6.25 ± 0.06c	0.35 ± 0.02c	5.46 ± 0.05c	5.78 ± 0.07e	51.51 ± 0.89c	6.76 ± 0.04g
VA14 × MSS	81.44 ± 2.16g	7.36 ± 0.05b	0.32 ± 0.01d	5.76 ± 0.06b	5.11 ± 0.06f	53.92 ± 0.82b	7.83 ± 0.08e
VA14 × SSS	81.07 ± 1.57g	8.16 ± 0.03a	0.28 ± 0.03f	6.12 ± 0.08a	4.38 ± 0.05i	54.52 ± 1.26a	8.75 ± 0.08b
Accession							
VA3	86.89 ± 1.65a	2.52 ± 0.02c	0.36 ± 0.02a	3.83 ± 0.05b	6.39 ± 0.04b	36.99 ± 0.99c	7.26 ± 0.05c
VA12	85.19 ± 1.86b	4.18 ± 0.04b	0.31 ± 0.01c	3.26 ± 0.08c	7.06 ± 0.03a	46.34 ± 1.14b	8.28 ± 0.07a
VA14	81.56 ±1.92c	7.25 ± 0.06a	0.32 ± 0.03b	5.78 ± 0.07a	5.09 ± 0.06c	53.32 ± 1.13a	7.78 ± 0.09b
Salinity stress (SS)							
NS	85.52 ± 1.58a	4.05 ± 0.03c	0.38 ± 0.04a	3.89 ± 0.06c	6.17 ± 0.07b	43.23 ± 1.08c	6.81 ± 0.05c
MSS	84.60 ± 1.49b	4.63 ± 0.05b	0.34 ± 0.02b	4.29 ± 0.08b	6.15 ± 0.06b	45.41 ± 1.15b	7.81 ± 0.09b
SSS	83.53 ± 1.74c	5.29 ± 0.06a	0.27 ± 0.03c	4.70 ± 0.07a	6.21 ± 0.08a	48.00 ±1.18a	8.70 ± 0.07a
Significance							
Accession	***	***	***	***	***	***	***
SS	***	***	***	***	***	***	***
Accession × SS	***	***	***	***	***	***	***

SS, Salinity stress; NS, No saline water; MSS, Moderate salinity stress, SSS, Severe salinity stress, Values are means of six replicates and different letters are differed significantly by Duncan Multiple Range Test ($P < 0.001$).

In the case of accession × salinity stress interaction, accession VA3 had the highest moisture content (86.16 g 100 g^{-1}) at no salinity stress condition. Both MSS and SSS reduced the moisture content at the lowest levels (81.07 and 81.44 g 100 g^{-1}) in accession VA14 that were followed by followed by accessions VA14 and VA14 under NS and SSS conditions, respectively. The highest protein content (8.16 g 100 g^{-1}) was recorded in accession VA14 under SSS followed by VA14 (7.36 g 100 g^{-1}) and VA14 (6.25 g 100 g^{-1}) under MSS and NS conditions, respectively. The lowest protein content (2.15 g 100 g^{-1}) was found in accession VA3 under nonsaline treatment, which was almost similar to VA3 under MSS (2.27 g 100 g-

^{1}). The fat contents in *A. tricolor* varied from 0.43 to 0.27 g 100 g^{-1}. The highest fat content (0.43 g 100 g^{-1}) was recorded in accession VA3 when no salinity stress was given to the plants whereas fat content in VA3 and VA12 was as low as 0.27 g 100 g^{-1} under SSS conditions. Accession VA3 also had the highest carbohydrate content (7.17 g 100 g^{-1}) when plants were treated with SSS. On the other hand, VA14 under MSS (5.11 g 100 g^{-1}) had the lowest carbohydrates content.

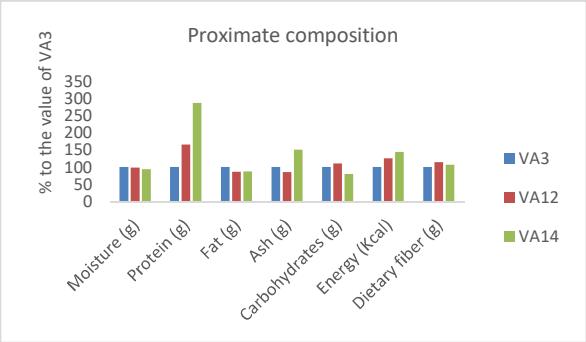

Fig. 1. Influence of salinity on proximate composition (g 100 g^{-1}) (% to the value of VA3) in three selected *A. tricolor* accessions.

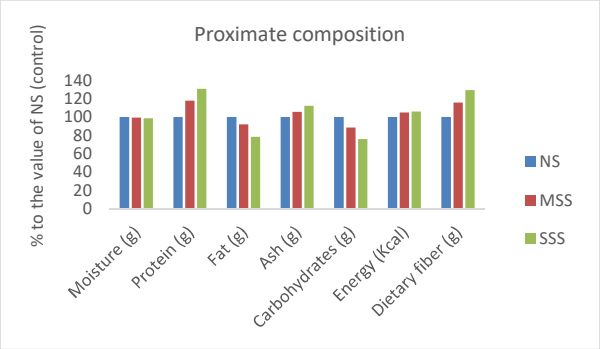

Fig. 2. Changes of proximate composition (g 100 g^{-1}) (% to the value of NS or control) in the leaves of *A. tricolor* accessions under three salinity stress levels. NS or control, no saline water; MSS, moderate salinity stress; and SSS, severe salinity stress.

The ash content in *A. tricolor* accessions varied (2.68 to 6.12 g 100 g^{-1}) under varying levels of salt stress. The highest ash content (6.12 g 100 g^{-1}) was recorded in accession VA14 at SSS conditions. The lowest as content (2.68 g 100 g^{-1}) was found in accession VA12 under non-saline control. The energy contents in *A. tricolor* plants ranged from 33.60 to 54.52 Kcal 100 g^{-1}. The accession VA14 exhibited the highest energy (54.52 Kcal 100 g^{-1}) under SSS followed by VA14 under MSS and NS or control, respectively. In contrast, the lowest energy was recorded in VA3 under NS or control. The energy was significantly increased to the increment of salinity stress in the following order: NS or control < MSS < SSS. The accession VA12 under SSS had the highest fiber content (9.24 g 100 g^{-1}) followed by VA14 under SSS (8.75 g 100 g^{-1}), VA12 under MSS (8.37 g 100 g^{-1}), VA3 under SSS (8.11 g 100 g^{-1}). Alternatively, VA3 under NS or control had the lowest fiber content (6.45 g 100 g^{-1}).

The interesting finding of this study is that responses of biochemical contents in different *A. tricolor* accessions were different. The accession, VA14 performed better in terms of protein, ash content, and energy content, respectively compared to the accession VA3. Similarly, the accession VA12 performed better in relation to carbohydrates and energy, respectively than the accession VA3 (Table 1). The maturity could have a great impact on the

moisture content of *A. tricolor* leaves. The moisture contents obtained in this investigation were in full agreement with the reports on sweet potato leaves by Sun *et al.* [175]. Fats are sources of omega-3 and omega-6 fatty acids. It helps in the digestion, absorption, and transport of fat-soluble vitamins A, D, E, and K. Sun *et al*. [175] observed similar results from sweet potato leaves where they mentioned that fat involved in the insulation of body organs and in the maintenance of body temperature and cell function.

As lower moisture contents of leaves are associated with higher dry matter, the salt-stressed plant yielded higher dry matter compared to control or NS. The highest contents of protein, ash, energy and dietary fiber at SSS conditions and the lowest values of these plant parameters in the control or NS appears that protein, ash, energy and dietary fiber contents in *A. tricolor* increased by salinity stress in a dose-dependent manner. The increment of protein, ash, energy and dietary fiber contents in *A. tricolor* at MSS and SSS could be contributed to human diet in the communities of saline prone area compared to non-saline area. Dietary fiber has a significant role in palatability, digestibility and remedy of constipation [151]. Vegetarian and poor people in many least developed Asian and African countries used *A. tricolor* as a source of protein. Plants cultivated in SSS had progressively higher energy than those of MSS and control or NS. However, these differences may not impact significantly on energy contribution to the human body as low amounts of this vegetable consumed in a day. Like other leafy vegetables, the low carbohydrate content of *A. tricolor* may not have a significant impact on carbohydrate contribution to the human body considering the low amount of vegetable uptake per day and a very high daily requirement for the human body.

A remarkable observation of this investigation is that the content of protein is increased with plants grown in higher doses of salinity. However, the trend of fat contents in plants under salinity treatment was just opposite to the contents of protein. It indicates that both salinity and accession had a complex influence on carbohydrate contents in *A. tricolor* plants. In an earlier study, Petropoulos *et al*. [117] demonstrated ameliorate response in carbohydrates, protein and fat content in *Cichorium spinosum* under salinity stress. Salt stress increased the protein, dietary fiber, energy, ash and carbohydrates content and decreased moisture and fat content of *A. tricolor* accessions. Therefore, amaranth produced saline prone area and coastal belt could contribute as a good source of protein and fiber in the human diet

Effects of salinity on mineral (macro and micro elements) composition in leaves

The mineral compositions of *A. tricolor* accessions significantly varied with varying levels of salinity stress and accession × salinity stress interactions (Table 2).

Among the tested accessions, the highest Ca, K, Fe, Mn, Cu and Zn contents were found in VA14. However, VA3 had the highest Mg content whereas the highest content of Na was recorded in VA12. In contrast, VA3 exhibited the lowest contents of Ca, K, Fe, Cu and Zn. Similarly, VA14 had the lowest Mg and Na content and VA12 showed the lowest Mn content. Accession VA14 exhibited 28%, 88%, 82%, 43%, 49% and 52% higher Ca, K, Fe, Mn, Cu and Zn content, respectively compared to VA3. Accession VA12 had 24% more Na content compared to VA3. (Fig. 3).

Table 2. Salinity stress on mineral composition (macro mg g^{-1} FW and micro µg g^{-1} FW nutrient elements) in the leaves of three selected *A. tricolor* accessions.

	Macroelements (mg g^{-1} FW)			Microelements (µg g^{-1} FW)				
Treatment	Ca	Mg	K	Fe	Mn	Cu	Zn	Na
Accession × Salinity stress (SS)								
VA3 × NS	2.05 ± 0.07h	2.55 ± 0.02e	4.58 ± 0.02d	10.26 ± 0.08i	10.23 ± 0.06i	0.98 ± 0.02i	10.58 ± 0.08i	62.55 ± 0.14i
VA3 × MSS	3.16 ± 0.05f	3.42 ± 0.03c	3.44 ± 0.01g	12.25 ± 0.09h	16.47 ± 0.04f	1.22 ± 0.01g	12.45 ± 0.07h	126.45 ± 0.21f
VA3 × SSS	4.26 ± 0.04c	4.72 ± 0.04a	2.25 ± 0.03i	14.62 ± 0.08f	21.31 ± 0.04b	1.76 ± 0.03e	16.35 ± 0.05d	246.55 ± 0.25b
VA12 × NS	2.37 ± 0.03g	2.50 ± 0.01e	4.33 ± 0.04e	13.35 ± 0.07g	11.22 ± 0.05h	1.12 ± 0.02h	13.13 ± 0.06g	74.63 ± 0.23h
VA12 × MSS	3.57 ± 0.07d	3.42 ± 0.05c	3.76 ± 0.03f	17.56 ± 0.05 d	13.55 ± 0.08g	1.58 ± 0.01f	15.63 ± 0.08e	148.94 ± 0.25d
VA12 × SSS	4.85 ± 0.06b	3.91 ± 0.06b	2.36 ± 0.02h	22.78 ± 0.09b	17.62 ± 0.06d	2.15 ± 0.02d	19.34 ± 0.09b	320.66 ± 0.26a
VA14 × NS	3.34 ± 0.05e	2.47 ± 0.02e	7.58 ± 0.04a	16.67 ± 0.08e	16.66 ± 0.07e	2.35 ± 0.03c	14.76 ± 0.08f	80.63 ± 0.28g
VA14 × MSS	3.62 ± 0.05d	2.86 ± 0.04d	6.11 ± 0.01b	18.53 ± 0.05c	19.43 ± 0.03c	3.25 ± 0.04b	17.65 ± 0.07c	132.45 ± 0.27e
VA14 × SSS	5.24 ± 0.06a	3.35 ± 0.03c	5.67 ± 0.03c	32.46 ± 0.07a	32.58 ± 0.05a	4.28 ± 0.02a	27.56 ± 0.09a	182.95 ± 0.29c
Accession								
VA3	3.16 ± 0.07c	3.57 ± 0.05a	3.42 ± 0.02c	12.38 ± 0.06c	16.00 ± 0.05b	1.32 ± 0.02c	13.13 ± 0.06c	145.18 ± 0.24b
VA12	3.60 ± 0.02b	3.28 ± 0.06b	3.49 ± 0.03b	17.90 ± 0.08b	14.13 ± 0.06c	1.62 ± 0.03b	16.03 ±0.08b	181.41 ± 0.28a
VA14	4.07 ± 0.03a	2.90 ± 0.01c	6.45 ± 0.02a	22.55 ± 0.09a	22.89 ± 0.05a	3.30 ± 0.01 a	19.99 ±0.08a	132.01 ± 0.27c
Salinity stress (SS)								
NS	2.58 ± 0.05c	2.51 ± 0.02c	5.50 ± 0.01a	13.43 ± 0.08c	12.70 ± 0.04c	1.49 ± 0.02c	12.82 ± 0.07c	72.60 ± 0.24c
MSS	3.45 ± 0.04b	3.24 ± 0.04b	4.44 ± 0.02b	16.11 ± 0.07b	16.48 ± 0.03b	2.02 ± 0.01b	15.25 ± 0.09b	135.94 ± 0.26b
SSS	4.78 ± 0.03a	3.99 ± 0.06a	3.43 ± 0.04c	23.29 ± 0.05a	23.84 ± 0.05a	2.73 ± 0.03a	21.08 ± 0.08a	250.06 ± 0.28a
Significance								
Accession	***	***	***	***	***	***	***	***
SS	***	***	***	***	***	***	***	***
Accession × SS	***	***	***	***	***	***	***	***

SS, Salinity stress; NS, No saline water; MSS, Moderate salinity stress, SSS, Severe salinity stress, Values are means of six replicates and different letters are differed significantly by Duncan Multiple Range Test ($P < 0.001$).

Across the salinity stresses, Ca, Mg, Fe, Mn, Cu, Zn and Na contents in leaves were sharply and significantly increased with the increment of salinity stress in the following order: NS or control < MSS < SSS. At MSS and SSS, the rate of the increment of Ca, Mg, Fe, Mn, Cu, Zn and Na were (8%, %, 11%, 16%, 38%, 19%, 64%) and (57%, 35%, 95%, 96%, 82%, 87%, 27%), respectively, over NS or control (Fig. 4). Further, it was noted that the severity of salinity stress leads to a significant reduction in K content in the following order: NS or control > MSS > SSS. In MSS and SSS, K reduced 19% and 25%, respectively over NS or control. (Fig. 4). SSS had the highest Ca, Mg, Fe, Mn, Cu, Zn and Na content while, the lowest Ca, Mg, Fe, Mn, Cu, Zn and Na content were demonstrated in NS or control. On the contrary, the highest K content was documented in NS or control and the lowest K content was observed in SSS.

Considering the accession × salinity stress interaction, the highest Ca content was noted in VA14 under SSS (5.24 mg g^{-1} FW) followed by VA12 under SSS and VA3 under SSS. In contrast, VA3 under NS or control (2.05 mg g^{-1} FW) displayed the lowest Ca content. Mg

content ranged from 2.47 to 4.72 mg g^{-1} FW. The highest Mg content was observed in VA3 under SSS (4.72 mg g^{-1} FW) followed by VA12 under SSS, VA12 under MSS and VA3 under MSS. In contrast, VA14, VA12 and VA3 under NS or control (2.47, 2.50 and 2.55 mg g^{-1} FW) displayed the lowest Mg content. The range of K content was 2.25 to 7.58 mg g^{-1} FW. VA14 under NS had the highest K content (7.58 mg g^{-1} FW) followed by VA14 under SSS and VA14 under MSS, while the lowest K content was noticed in VA3 under NS (2.55 mg g^{-1} FW). The Fe content ranged from 10.26 to 32.46 µg g^{-1} FW. The highest Fe content was observed in VA14 under SSS (32.46 µg g^{-1} FW), whereas, VA3 under NS (10.26 µg g^{-1} FW) exhibited the lowest Fe content. Mn content ranged from 10.23 to 32.58 µg g^{-1} FW. Accession VA14 under SSS exhibited the highest Mn content (32.58 µg g^{-1} FW), while, VA3 under NS had the lowest Mn content (10.23 µg g^{-1} FW). Accession VA14 under SSS had the highest Cu content (4.28 µg g^{-1} FW). In contrast, the lowest Cu content (0.98 µg g^{-1} FW) was recorded in VA3 under NS. Zn content ranged from 10.58 to 27.56 µg g^{-1} FW. Accession VA14 under SSS showed the highest Zn content (27.56 µg g^{-1} FW), whereas, the lowest Zn content (10.58 µg g^{-1} FW) was reported on VA3 under NS. The highest Na content was detected in VA12 under SSS (320.66 µg g^{-1} FW) which ranged from 62.55 to 320.66 µg g^{-1} FW. The lowest Na content (62.55 µg g^{-1} FW) was recorded in VA3 under NS.

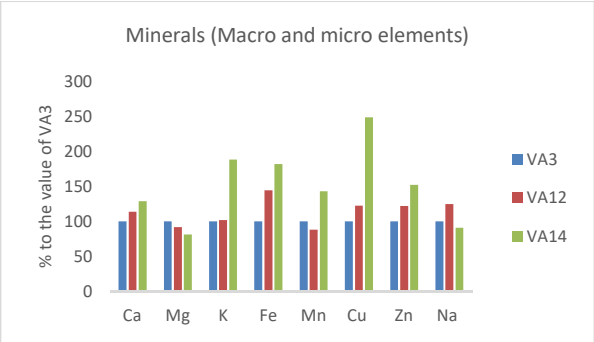

Fig. 3. Minerals (macro mg g^{-1} and micro µg g^{-1} nutrient elements) contents (% to the value of VA3) in the leaves of three selected *A. tricolor* accessions.

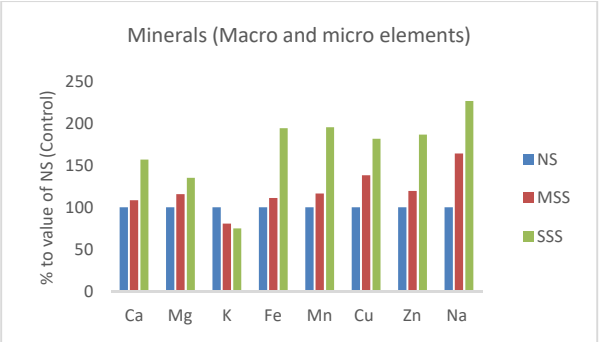

Fig. 4. Comparison of minerals (macro mg g^{-1} and micro µg g^{-1} nutrient elements) (% to the value of NS or control) contents in *A. tricolor* leaves under three salinity levels. NS or control, no salinity stress; MSS, moderate salinity stress; and SSS, severe salinity stress.

We observed that salinity stress influences the mineral compositions of *A. tricolor* accessions. Among the tested accessions, VA14 could be consider as Ca, K, Fe, Mn, Cu and Zn enrich accession, VA3 as Mg and VA12 as Na enrich accessions (Table 2). In *A. tricolor*, iron and zinc content is higher than that of the leaves of cassava [247] and beach pea [248].

Similarly, Jimenez-Aguiar and Grusak [246] reported high Fe, Mn, Cu and Zn (fresh weight basis) in different *A. spp.* including *A. tricolor*. They also reported that Amaranths had higher Zn content than black nightshade, spinach and kale; more Fe and Cu content than kale. Across salinity stress, Ca, Mg, Fe, Mn, Cu, Zn and Na content were sharply and significantly increased with the increment of salinity stress in the following order: NS or control < MSS < SSS. In contrast, it was noted that the severity of salinity stress leads to a significant reduction in K content in the following order: NS or control > MSS > SSS (Table 2). These results were fully agreed with the findings of Petropoulos *et al*. [117] that observed similar increment in Ca, Mg, Fe, Mn, Zn and Na and decrement in K content in *C. spinosum* leaves. They mentioned the high content of Na should be attributed to fertilizer application and salinity treatments and suggested that the species uses Na accumulation as a means to alleviate adverse effects of salinity. With the severity of salinity stress, all the macro and micro elements except K showed increasing trend, while K showed the declining trend with the severity salinity stress. For this, amaranth cultivated in salinity prone area and coastal belt could contribute as a good source of minerals in human diet compared to normal cultivation practices.

Salinity stress enhances beta-carotene, ascorbic acid, TPC, TFC and TAC in A. tricolor leaves

Total polyphenol content (TPC), beta-carotene, total flavonoid content (TFC), ascorbic acid, and total antioxidant capacity (TAC) in *A. tricolor* leaves were significantly affected by accession, salt concentration and accession × salt concentration interactions (Table 3).

Within accessions, TPC, beta-carotene, TAC (DPPH), ascorbic acid, and TAC ($ABTS^+$) was the highest in VA14 and VA12 had the highest TFC followed by VA14. Accession VA12 exhibited the lowest TAC (DPPH), beta-carotene, TPC, and TAC ($ABTS^+$). The VA14 had 74%, 10.07-fold, 46%, 7%, and 15% increase in TPC, beta-carotene, TAC (DPPH), ascorbic acid, and TAC ($ABTS^+$), respectively compared to VA3. Accession VA12 exhibited 3.9-fold and 190% increase in ascorbic acid and TFC, respectively, compared to VA3. (Fig. 5). In our study, beta-carotene, ascorbic acid, TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) were significantly increased with the increment of salinity stress in the following order: NS < MSS < SSS. In MSS and SWS, beta-carotene, ascorbic acid, TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) were increased in (56%, 31%, 15%, 16%, 25% and 16%) and (112%, 115%, 39%, 30%, 58% and 47%) compared to NS, respectively (Fig. 6). The highest beta-carotene, ascorbic acid, TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) were noticed in SSS while, the lowest beta-carotene, ascorbic acid, TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) were observed in NS. Regarding the interaction of accession × salinity stress, VA14 under SSS exhibited the highest

beta-carotene, ascorbic acid, TPC, TAC (DPPH) and TAC ($ABTS^{+}$), while VA12 under SSS had the highest TFC. In contrast, the lowest beta-carotene, TPC, TAC (DPPH) and TAC ($ABTS^{+}$) was observed in VA12 under NS, while VA3 under NS showed the lowest ascorbic acid and TFC. Only, ascorbic acid was significantly increased to the increment of salinity stress in all the accessions in the following order: NS < MSS < SSS. Higher beta-carotene was observed in VA14 under MSS, VA12 under SSS, VA3 under SSS, VA14 under NS, while a high ascorbic acid was recorded in VA14 under MSS and VA14 under NS. Higher TPC was found in VA3 under SSS and VA3 under MSS, whereas, VA12 under MSS, VA14 under SSS and VA12 under NS had a high TFC. VA14 under MSS, VA3 under SSS, VA3 under MSS and VA3 under N showed a high TAC (DPPH), while VA14 under MSS, VA3 under SSS and VA12 under SSS had a high TAC ($ABTS^{+}$).

Table 3. Salinity effects on antioxidant phytochemicals and antioxidant capacity in three selected *A. tricolor* accessions

Treatment	beta-carotene (mg kg^{-1})	Ascorbic acid (mg kg^{-1})	Total polyphenol content (GAE mg kg^{-1} dw)	Total flavonoid content (RE mg kg^{-1} dw)	Total antioxidant capacity (DPPH) (TEAC mg kg^{-1} dw)	Total antioxidant capacity ABTS+) (TEAC mg kg^{-1} dw)
Accession × Salinity stress (SS)						
VA3 × NS	8.28 ± 0.09g	165.74 ± 2.15i	28.25 ± 0.24e	110.45 ± 1.47i	32.56 ± 0.15d	55.56 ± 0.37g
VA3 × MSS	8.74 ± 0.11e	212.86 ± 2.47h	30.63 ± 0.31c	129.85 ±1.52h	33.45 ± 0.24c	58.75 ± 0.45e
VA3 × SSS	10.26 ± 0.12c	286.63 ± 3.12g	32.45 ± 0.42b	154.17 ± 2.02g	35.42 ± 0.16b	63.52 ± 0.57c
VA12 × NS	7.78 ± 0.15h	984.65 ± 3.51f	20.26 ± 0.33i	340.65 ± 2.48d	23.52 ± 0.27h	52.35 ± 0.62h
VA12 × MSS	8.44 ± 0.07f	1052.74 ± 3.24e	22.49 ± 0.38h	385.96 ± 3.07b	24.55 ± 0.23g	56.38 ± 0.53f
VA12 × SSS	10.32 ± 0.14c	1225.53 ± 2.87d	26.36 ± 0.54f	422.54 ± 1.95a	26.88 ± 0.24f	61.62 ± 0.28d
VA14 × NS	10.18 ± 0.08d	1645.15 ± 1.58c	25.53 ± 0.37g	280.64 ± 1.25f	28.35 ± 0.17e	56.31 ± 0.41f
VA14 × MSS	15.89 ± 0.16b	2156.17 ± 2.62b	29.6 ± 0.29d	325.88 ± 1.27e	35.45 ± 0.16b	65.76 ± 0.62b
VA14 × SSS	21.46 ± 0.13a	3563.52 ± 2.57a	35.52 ± 0.26a	364.37 ± 2.22c	44.85 ± 0.20a	82.55 ± 0.82a
Accession						
VA3	9.14 ± 0.11c	221.62 ± 2.08c	30.44 ± 0.23a	131.49 ± 2.18c	33.81 ± 0.17b	59.27 ± 0.49b
VA12	8.58 ± 0.06b	1087.44 ± 2.49b	23.04 ± 0.28b	383.12 ± 1.67a	24.98 ± 0.26c	56.78 ± 0.27c
VA14	15.82 ± 0.05a	2454.67 ± 1.68a	30.22 ± 0.19a	323.63 ± 2.35b	36.22 ± 0.22a	68.21 ± 0.37a
Salinity stress (SS)						
NS	8 .75 ± 0.15c	931.47 ± 3.01c	24.68 ± 0.17c	243.89 ± 1.37c	28.15 ± 0.23c	54.74 ± 0.28c
MSS	11.46 ± 0.16b	1140.84 ± 3.48b	27.57 ± 0.24b	280.55 ± 1.92b	31.15 ± 0.19b	60.31 ± 0.34b
SSS	14.27 ± 0.17a	1691.62 ± 2.68a	31.44 ± 0.18a	313.78 ± 1.83a	35.72 ± 0.17a	69.23 ± 0.28a
Significance						
Accession	***	***	***	***	***	***
SS	***	***	***	***	***	***
Accession × SS	***	***	***	***	***	***

SS, Salinity stress; NS, No saline water; MSS, Moderate salinity stress, SSS, Severe salinity stress, Values are means of six replicates and different letters are differed significantly by Duncan Multiple Range Test ($P < 0.001$).

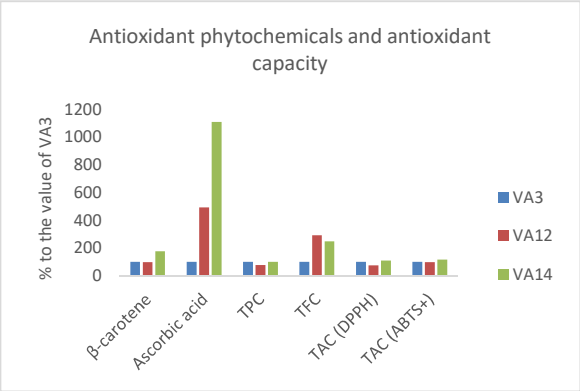

Fig. 5. Response of antioxidant phytochemicals and antioxidant activities (% to the value of VA3) in three selected *A. tricolor* accessions; beta-carotene (mg kg^{-1}), Ascorbic acid (mg kg^{-1}), TPC, Total polyphenol content (GAE mg kg^{-1} dw); TFC, Total flavonoid content (RE mg kg^{-1} dw); TAC (DPPH), Total antioxidant capacity (DPPH) (TEAC mg kg^{-1} dw); TAC ($ABTS^{+}$), Total antioxidant capacity ($ABTS^{+}$) (TEAC mg kg^{-1} dw)

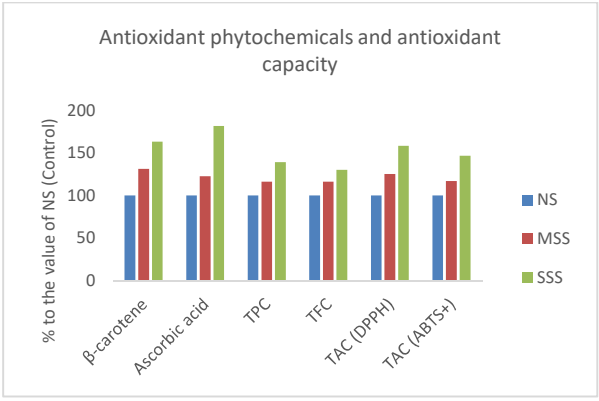

Fig. 6. Response of antioxidant phytochemicals and antioxidant capacity (% to the value of NS or Control) under three salinity levels: NS or Control (No saline water), MSS (Moderate salinity stress), SSS (Severe salinity stress) in three selected *A. tricolor* accessions; beta-carotene (mg kg^{-1}), Ascorbic acid (mg kg^{-1}), TPC, Total polyphenol content (GAE mg kg^{-1} dw); TFC. Total flavonoid content (RE mg kg^{-1} dw); TAC (DPPH), Total antioxidant capacity (DPPH) (TEAC mg kg^{-1} dw); TAC ($ABTS^{+}$), Total antioxidant capacity ($ABTS^{+}$) (TEAC mg kg^{-1} dw)

One of the interesting findings of our study is that salinity stresses 50 mM and 100 mM NaCl concentrations significantly improved protein, ash, energy, dietary fiber, Ca, Mg, Fe, Mn, Cu, Zn, Na, beta-carotene, ascorbic acid, total polyphenol content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC) (DPPH) and total antioxidant capacity (TAC) ($ABTS^{+}$) (Table 1, 2 and 3) in leaves of *A. tricolor* compared to control condition. control. Salt-stressed *A. tricolor* leaves also showed remarkable increment in protein, ash, energy, dietary fiber, minerals and functional antioxidant phytochemicals compared to normal cultural condition (Fig. 2, 4 and 6). To the best of our knowledge, this is the first report of remarkable and progressive improvement of the proximate, nutritional and functional antioxidant phytochemicals contents in *A. tricolor* under salinity stresses compared to non-saline soil conditions.

An important finding of the current study is that beta-carotene, ascorbic acid, total polyphenol content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) of *A. tricolor* leaves were significantly augmented by the salt stress at certain level (Table 3). These important phytochemicals content were remarkably influenced by the accessions and accession × salt concentration interactions. The accessions VA14 could be consider as TPC, beta-carotene, TAC, ascorbic acid, antioxidant enrich accession and VA12 as flavonoid enrich accession. In the present study, we found great variations in the tested accessions in terms of TPC, beta-carotene, TFC, TAC (DPPH) and TAC ($ABTS^{+}$) in different salinity levels (Table

3). Similarly, Alam *et al*. [122] reported pronounced variations in TFC, TPC, and TAC in different purslane accessions.

In our study, beta-carotene, ascorbic acid, TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) were significantly increased with the increment of salinity stress in the following order: NS < MSS < SSS. VA14 under SSS exhibited the highest beta-carotene, ascorbic acid, TPC, TAC (DPPH) and TAC ($ABTS^+$), while VA12 under SSS had the highest TFC. In contrast, the lowest beta-carotene, TPC, TAC (DPPH) and TAC ($ABTS^+$) was observed in VA12 under NS, while VA3 under NS showed the lowest ascorbic acid and TFC. When plants fall under salinity stress, reactive oxygen species (ROS) are produced as a result of oxidative stress. ROS induces harmful effects on plant cells. As a result, defenses against ROS are activated by generation of an array of nonenzymatic antioxidants such as ascorbic acid (AsA) and beta-carotene [97]. Salinity stress induces mevalonic acid pathway which are responsible for biosynthesis of abscisic acid (ABA) from carotenoids to counteract the osmotic stress and regulate normal plant growth and development [246]. Therefore, salinity stress enhances the accumulation of beta-carotene due to induction of ABA. AsA and αtocopherols play a crucial role in quenching intermediate/excited reactive forms of oxygen molecule directly or through catalysis of enzymes. AsA scavenges ROS (OH, SOR and $^1O_2$ directly and reduces H_2O_2 to water through ascorbate peroxidase reaction [206]. Antioxidant ascorbate and total carotenoid had vital role in counterbalancing oxidative stress and manipulating homeostasis of ROS in plants [237]. Wouyou *et al*. [245] observed ameliorate response of vitamin A and vitamin C at 90 mM NaCl concentration in *Amarantus cruentus* leaves. Similarly, Petropoulos *et al*. [117] found an elevated response to phenolics, flavonoids and antioxidant activity with the increase in salt stress in *Cichorium spinosum*. Alam *et al*. [118] observed that in purslane, different doses of salt concentrations increased total polyphenol content (TPC); total flavonoid content (TFC) and FRAP activity by 8–35%, 35% and 18–35%, respectively. Lim et al. [119] reported that buckwheat treated with 10, 50, and 100 mM after 7 d of cultivation had 57%, 121% and 153%, respectively, higher phenolic content than that of the control. Ahmed *et al*. [247] reported the increment of phenolics and TAC (FRAP) with increasing NaCl concentrations in barley. In contrast, Neffati *et al*. [248] found decrement in polyphenols and TAC (DPPH) with increasing NaCl concentrations in coriander. The increment of TPC, TFC and TAC of *A. tricolor* in response to salinity stress may be due to increase in major phenolic compounds like salisylic acid, gallic acid, vanilic acid, *p*-hydroxybenzoic acid, chlorogenic acid, *m*-coumaric acid, *trans*-cinnamic acid, iso-quercetin and rutin [212]. Previous studies have shown that biotic and

abiotic stress stimulated phenylpropanoid pathway which accelerated the generation of most phenolic compounds [249, 250]. Stress-plants induce endogenous plant hormones like jasmonic acid and its methylated derivate (methyl jasmonic acid) [251]. These hormones sequentially induce phenylpropanoid pathway enzymes, including phenylalanine ammonia lyase (PAL) [252]. These enzymes accumulated the phenolic compounds.

Correlation coefficients among antioxidant phytochemicals and antioxidant activity

Results of correlation studies are presented in Table 4. beta-carotene showed highly significant interrelationships with ascorbic acid, TAC (DPPH), TAC ($ABTS^+$) while, this trait had significant associations with TPC and TFC. Similarly, ascorbic acid revealed significant ascorbic acid played a vital role in the antioxidant activity of *A. tricolor*. TPC, TFC, TAC (DPPH) significantly interrelated among each other. The beta-carotene showed highly significant interrelationships with ascorbic acid, TAC (DPPH), TAC ($ABTS^+$) while, this trait had significant associations with TPC and TFC. Similarly, ascorbic acid revealed significant interrelationships with TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) (Table 4). ascorbic acid interrelationships with TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$). Both beta-carotene and played a vital role in the antioxidant activity of *A. tricolor*. TPC, TFC, TAC (DPPH) significantly interrelated among each other. Polyphenols and flavonoids of *A. tricolor* leaf establishing strong antioxidant activity. Alam *et al*. [118] reported the significant correlation of carotenoids, TPC, TFC with TAC (FRAP) in salt-stressed purslane.

Table 4. Correlation coefficient for antioxidant phytochemical and antioxidant capacity in three selected *A. tricolor* accessions

	beta-carotene (mg kg^{-1})	Ascorbic acid (mg kg^{-1})	Total polyphenol content (GAE mg kg^{-1} dw)	Total flavonoid content (RE mg kg^{-1} dw)	Total antioxidant capacity (DPPH) (TEAC mg kg^{-1} dw)	Total antioxidant capacity ABTS+) (TEAC mg kg^{-1} dw)
beta-carotene		0.94**	0.68*	0.71*	0.82**	0.97**
AsA			0.66*	0.72*	0.76*	0.75*
TPC				0. 77*	0.95**	0.85**
TFC					0.84**	0.83**
TAC (DPPH)						0.96**

AsA, Ascorbic acid; TPC, Total polyphenol content; TFC, Total flavonoid content; TAC (DPPH), Total antioxidant capacity (DPPH); TAC ($ABTS^+$), Total antioxidant capacity ($ABTS^+$); *significant at 5% level, ** significant at 1% level, (n = 6)

In conclusion, a significant increment in protein, ash, energy, dietary fiber, carbohydrates, Ca, Mg, Fe, Mn, Cu, Zn, Na, beta-carotene, ascorbic acid, TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) in *A. tricolor* leaves were observed under salinity stress. All the nutritional values of *A. tricolor* leaves under MSS and SSS remarkably high compared to corresponding control or NS values which could be a valuable food source in modern diets and contribute considerably to human health. Furthermore, salt-stress also enhanced the contents of protein, ash, energy, dietary fiber, Ca, Mg, Fe, Mn, Cu, Zn, Na, beta-carotene, ascorbic acid,

TPC, TFC in leafy vegetables *A. tricolor*. The vitamins, phenolics and flavonoids showed a good antioxidant activity due to positive and significant interrelationships with TAC. Our results suggest that *A. tricolor* cultivated under salinity stress could be contributed to a high nutritional quality of the final product in terms of nutrients, minerals, vitamins and antioxidant profiles. Therefore, *A. tricolor* could be considered as a promising alternative crop for farmers, especially in salinity-prone areas and the coastal belts in tropical and sub-tropical countries.

Abstract

Impact of salinity stress were investigated in three selected *A. tricolor* accessions in terms of nutrients, dietary fiber, minerals, antioxidant phytochemicals and total antioxidant activity in leaves. Salinity stress enhanced biochemical contents and antioxidant activity in *A. tricolor* leaves. Protein, ash, energy, dietary fiber, minerals (Ca, Mg, Fe, Mn, Cu, Zn, and Na), beta-carotene, ascorbic acid, total polyphenol content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC) (DPPH and $ABTS^{+}$) in leaves were increased by 18%, 6%, 5%, 16%, 9%, 16%, 11%, 17%, 38%, 20%, 64%, 31%, 22%, 16%, 16%, 25% and 17%, respectively at 50 mM NaCl concentration and 31%, 12%, 6%, 30%, 57%, 35%, 95%, 96%, 82%, 87%, 27%, 63%, 82%, 39%, 30%, 58% and 47%, respectively at 100 mM NaCl concentration compared to control condition. Contents of vitamins, polyphenols and flavonoids showed a good antioxidant activity due to positive and significant interrelationships with total antioxidant capacity. It revealed that *A. tricolor* can tolerate a certain level of salinity stress without compromising the nutritional quality of the final product. This report for the first time demonstrated that salinity stress at certain level remarkably enhances nutritional quality of the leafy vegetable *A. tricolor*. Taken together, our results suggest that *A. tricolor* could be a promising alternative crop for farmers in salinity prone areas- in the tropical and sub-tropical regions with enriched nutritional contents and antioxidant activity.

3.3.2 Salinity stress enhances color parameters, bioactive leaf pigments, vitamins, polyphenols, flavonoids and antioxidant activity in selected *Amaranthus* leafy vegetables

Purpose of the study

Salinity stress intensifies the overproduction of reactive oxygen species (ROS) that interfere with normal cellular metabolism and result in oxidative damage by oxidizing proteins, lipids, DNA and other cellular macromolecules [253]. Plants show variable adaptation processes, such as the enclosure of stomata, metabolic adjustment, toxic ion homeostasis, and osmotic adjustment to compensate for osmotic stress [112]. Non-enzymatic compatible solutes and antioxidants, such as proteins, carbohydrates, ascorbic acid (AsA), beta-carotene, carotenoids, phenolic compounds and flavonoids, and enzymatic antioxidants, such as superoxide dismutase (SOD), peroxidase (GPOX), catalase (CAT) and AsA peroxidase (APX), have played vital roles in the ROS detoxification system of stressed plants [253].

Salt stress elevated ascorbic acid, phenolics, flavonoids and antioxidant activity and reduced the chlorophyll pigments, in *Cichorium spinosum* [117]. Alam *et al*. [118] observed that different levels of salinity treatment resulted in 8–35% increases in TPC; about 35% increase in TFC; and 18–35% increases in FRAP activity in purslane. Lim *et al*. [119] reported that buckwheat treated with 10, 50, 100, and 200 mM NaCl concentrations resulted in an increase of phenolic compounds and carotenoids in the sprouts compared to the control (0 mM). The phenolic contents of sprouts treated with 10, 50, and 100 mM NaCl after 7 d of cultivation were 57%, 121%, and 153%, respectively, higher than that of the control.

The results of these studies show that salt stress elevated these compounds in many leafy vegetables. We hypothesize that salinity stress can enhance the leaf pigments, ascorbic acid (AsA), carotenoids, polyphenols, flavonoids and antioxidant activity of the *A. tricolor* leafy vegetable. *A. tricolor* is a salt-tolerant genotype, and it can tolerate up to 200 mM NaCl [254]. To our knowledge, there is no information on the response of *Amaranthus tricolor* to salinity stress and its effects on antioxidant leaf pigments, carotenoids, vitamins, phenolics, flavonoids and antioxidant activity. In our previous studies [143, 149-151, 160-162, 173] we selected some enriched antioxidants and high yield potential genotypes. Therefore, this study aimed to examine the *A. tricolor* genotypes selected in response to salinity stress in terms of antioxidant leaf pigments, carotenoids, vitamins, phenolics, flavonoids and antioxidant activity.

Materials and methods

Experimental site, Plant materials and experimental conditions

The methods were used as previous chapter. At 35 DAS the leaves of *Amaranthus tricolor* were harvested.

Leaf color measurement

The color parameters L*, a* and b* were measured by a color meter (TES-135A, Plus, Taiwan). The value of L* indicates lightness, a* indicates the degree of red (+a*) or green (-a*) color, and b* indicates yellow (+b*) or blue (-b*) color. The C* value expressed as chroma indicates leaf color intensity calculated as Chroma $C^* = (a^2 + b^2)^{1/2}$.

Determination of betacyanin and betaxanthin content

Betacyanin and betaxanthin content were measured following the procedure described in the previous chapter

Determination of chlorophyll and total carotenoids

Chlorophyll and total carotenoids were measured following the procedure described in the previous chapter

Determination of beta-carotene and ascorbic acid

Beta-carotene and ascorbic acid content were measured following the procedure described in the previous chapter

Extraction of samples for TPC, TFC and TAC

Samples were extracted following the procedure described in the previous chapter

Estimation of beta-carotene, TPC, TFC and TAC

TPC, TFC and TAC were measured following the procedure described in the previous chapter

Statistical Analysis

Data were analyzed following the methods of previous chapter

Results and discussion

Color parameters and leaf pigments

Salinity stress significantly affected the color parameters and leaf pigments of *A. tricolor* genotypes, different salinity levels and genotype × salinity stress interactions as presented in Table 1 and Table 2. Of all the genotypes, VA3 exhibited the highest L, a*, b*, chroma, betacyanin, betaxanthin, betalain, and total carotenoids, while VA14 had the highest chlorophyll a, chlorophyll b, and total chlorophyll. Similarly, Alam *et al*. [118] reported variations in total carotenoid contents in different purslane accessions under salinity stress. In contrast, genotype VA12 showed the lowest betacyanin, betaxanthin, betalain, chlorophyll a, chlorophyll b and total chlorophyll, while genotype VA14 exhibited the lowest L and total carotenoids. The lowest a*, b* and chroma were obtained from both genotypes, VA12 and

VA14. Genotype VA3 had the highest red and yellow pigmentations [highest redness (a* = 18); highest yellowness (b* = 5.62) value; highest lightness (L = 38.32)], while VA14 and VA12 showed the lowest red and yellow pigmentations [lowest redness (a* = 13.81); lowest yellowness (b* = 3.82 and 3.96); lowest lightness (L = 33.47 and 34.78)]. Color is one of the most important parameters for consumers and plays a crucial role in decision making, preference and acceptability of the product and may also be considered as an indicator to estimate the antioxidant properties of the leafy vegetables [259]. The highest redness and yellowness values recorded in VA3 could be expected, since it is characterized by high amounts of pigments (anthocyanins, carotenoids, betacyanin, betaxanthin and betalain) involved in leaf pigmentation [259]. It is clear that VA3 and VA12 were antioxidant-enriched genotypes based on evaluations of the genotypes using color parameters and pigments.

Table 1. Salinity effect on leaf color parameters in three selected *A. tricolor* genotypes

Treatment	L*	a*	b*	Chroma
Genotype × Salinity stress (SS)				
VA3 × NS	36.43±0.29c	15.75±0.18c	4.95±0.06c	16.52±0.13c
VA3 × MSS	37.46±0.28b	17.65±0.19b	5.60±0.05b	18.52±0.18b
VA3 × SSS	41.06±0.17a	20.60±0.16a	6.33±0.08a	21.57±0.16a
VA12 × NS	34.13±0.39f	13.49±0.42f	3.34±0.05f	13.90±0.12e
VA12 × MSS	34.75±0.234e	13.77±0.26f	3.97±0.08e	14.33±0.15e
VA12 × SSS	35.47±0.26d	14.18±0.21e	4.16±0.04d	14.78±0.17e
VA14 × NS	32.45±0.21h	12.59±0.24h	3.88±0.09f	13.17±0.15f
VA14 × MSS	33.33±0.28g	13.92±0.15g	3.89±0.07e	14.45±0.08e
VA14 × SSS	34.65±0.24e	14.94±0.31d	4.11±0.02d	15.49±0.09d
Genotype				
VA3	38.32±0.18a	18.00±0.16a	5.62±0.08a	18.87±0.21a
VA12	34.78±0.25b	13.81±0.15b	3.82±0.05b	14.34±0.10b
VA14	33.47±0.22c	13.81±0.17b	3.96±0.06b	14.37±0.12b
Salinity stress (SS)				
NS	34.34±0.14c	13.94±0.21c	4.06±0.04c	14.53±0.14c
MSS	35.18±0.16b	15.11±0.18b	4.49±0.03b	15.77±0.19b
SSS	37.06±0.13a	16.57±0.16a	4.87±0.05a	17.28±0.18a
Significance				
Genotype	***	***	***	***
SS	***	***	***	***
Genotype × SS	***	***	***	***

SS, Salinity stress; NS, No saline water; MSS, Moderate salinity stress, SSS, Severe salinity stress, L*, Lightness; a*, Redness/greenness; b*, Yellowness/blueness; Values are means of six replicates and different letters are differed significantly by Duncan Multiple Range Test ($P < 0.001$).

Within salinity stress, L, a*, b*, chroma, betacyanin, betaxanthin, betalain, and total carotenoids increased remarkably {(no saline water (NS) < moderate salinity stress (MSS) < severe salinity stress (SSS)}, while chlorophyll a, chlorophyll b, and total chlorophyll declined significantly with severe salinity stress (NS > MSS > SSS). In MSS and SSS, the increases in the L, a*, b*, chroma, betacyanin, betaxanthin, betalain and total carotenoids were 2%, 12%, 13%, 12%, 10% 10%, 10%, and 37% and 13%, 31%, 28%, 31%, 18% 29%, 24%, and 85%, respectively, while the decreases in chlorophyll a, chlorophyll b and total chlorophyll contents were 3%, 13%, and 7% and 12%, 18% and 14%, respectively, compared to the NS (Fig. 1). Petropoulos *et al*. [117] observed reductions in the chlorophyll pigment content with the

severity of salinity stress in *Cichorium spinosum*. Lim *et al*. [119] observed a continuous increase in the level of carotenoids in response to all the NaCl concentrations tested. They reported the greatest difference between the carotenoid content with 50 or 100 mM NaCl, which was twice as high as that of the control sprouts, while treatment with 10 or 200 mM NaCl resulted in a 40% increase in carotenoids. In contrast, Alam *et al*. [118] reported both an increase and decrease in total carotenoid contents in different accessions of purslane with the severity of salinity stress.

Table 2. Salinity effect on antioxidant leaf pigments in three selected *A. tricolor* genotypes.

Treatment	betacyanin (ng g^{-1} FW)	betaxanthin (ng g^{-1} FW)	Betalain (ng g^{-1} FW)	Chl a (µg g^{-1} FW)	Chl b (µg g^{-1} FW)	Total chl (µg g^{-1} FW)	Total carotenoids (mg 100 g^{-1} FW)
Genotype × Salinity stress (SS)							
VA3 × NS	501.74±0.62e	505.35±0.38f	1007.09±1.12f	305.29±1.20d	228.59±0.24b	533.87±1.02d	67.46 ± 0.08ef
VA3 × MSS	552.74±0.84c	556.80±0.42e	1109.53±0.75e	233.41±1.08e	173.20±0.12e	406.61±0.88e	92.75 ± 0.09b
VA3 × SSS	592.70±0.54a	652.33±0.47a	1245.03±1.12a	159.35±0.98f	107.73±0.15f	267.08±0.75f	124.84 ± 0.07a
VA12 × NS	234.33±0.25h	228.66±0.28i	462.99±0.59i	132.45±0.45g	72.55±0.24g	205.00±0.59g	66.58 ± 0.13g
VA12 × MSS	262.62±0.47g	258.32±0.42h	520.95±0.87h	82.72±1.03h	53.75±0.22h	136.47±0.68h	75.83 ± 0.08d
VA12 × SSS	286.92±0.56f	276.38±0.62g	563.30±0.95g	55.64±0.88i	35.51±0.26i	91.15±0.88i	87.54 ± 0.11c
VA14 × NS	538.48±0.54d	582.49±0.24d	1120.97±1.02d	515.04±1.04a	252.44±0.34a	767.48±0.51a	56.53 ± 0.11i
VA14 × MSS	552.52±0.62c	595.79±0.35c	1148.31±1.13c	497.09±0.78b	219.99±0.24c	717.08±0.62b	64.53 ± 0.08h
VA14 × SSS	576.55±0.54b	612.56±0.25b	1189.10±1.26b	452.41±0.63c	207.63±0.11d	660.03±0.55c	72.92 ± 0.15e
Genotype							
VA3	555.85±0.35a	596.94±0.38a	1152.79±1.02a	232.68±1.12b	169.84±0.09b	402.52±0.36b	95.02 ± 0.13a
VA12	261.29±0.62c	254.45±0.52c	515.75±0.96c	90.27±0.89c	53.94±0.15c	144.21±0.52c	76.65 ± 0.08b
VA14	549.06±0.45b	571.49±0.42b	1120.55±0.75b	488.18±0.77a	226.69±0.14a	714.87±0.58a	64.66 ± 0.09c
Salinity stress (SS)							
NS	424.85±0.38c	438.83±0.28c	863.69±0.84c	317.59±1.05a	184.53±0.25a	502.12±0.75a	63.52 ± 0.09c
MSS	455.96±0.75b	470.30±0.37b	926.26±0.88b	271.07±0.59b	148.98±0.32b	420.05±0.59b	77.70 ± 0.07b
SSS	485.39±0.42a	513.75±0.64a	999.15±0.79a	222.47±0.58c	116.95±0.23c	339.42±0.46c	95.10 ± 0.08a
Significance							
Genotype	***	***	***	***	***	***	***
SS	***	***	***	***	***	***	***
Genotype × SS	***	***	***	***	***	***	***

SS, Salinity stress; NS, No saline water; MSS, Moderate salinity stress, SSS, Severe salinity stress, Chl a, chlorophyll a; Chl b, chlorophyll b; Total chl, Total chlorophyll; Values are means of six replicates and different letters are differed significantly by Duncan Multiple Range Test ($P < 0.001$).

An examination of the interaction of genotype × salinity stress indicated that VA3 under SSS exhibited the highest L, a*, b*, chroma, betacyanin, betaxanthin, betalain, and total carotenoids, and VA14 under NS had the highest chlorophyll a, chlorophyll b, and total chlorophyll. In contrast, the lowest L, a*, b*, chroma and total carotenoids were measured in VA14 under NS. The lowest betacyanin was recorded in VA12 under NS, and the lowest betaxanthin and betalain were obtained from VA12 under MSS. The lowest chlorophyll a, chlorophyll b and total chlorophyll contents were observed in VA12 under SSS. A higher L, a*, b*, and chroma were recorded in VA3 under NS and VA3 under MSS. High betacyanin was observed in VA3 under MSS and VA14 under SSS. Genotype VA14 under MSS and VA14 under SSS had high betaxanthin and betalain contents. VA14 under MSS exhibited a higher chlorophyll a and total chlorophyll content, while VA3 under NS and VA14 under MSS showed a high chlorophyll b content. Salinity stress affected plant growth and development through osmotic stress on the plants, reducing the water potential, decreasing the stomatal conductivity, which restricts the CO_2 influx to the leaves, and an unfavorable CO_2/O_2 ratio in the chloroplasts, reducing photosynthesis. To overcome salt stress, plants tend to accumulate

compatible solutes and antioxidants such as leaf pigments and carotenoids [255-257] that decrease the cytoplasmic osmotic potential, enabling water absorption [258]. As a result, plants can adapt to salinity stress and continue normal growth. Unlike other biotic and abiotic stresses, salinity stress induces the biosynthesis of abscisic acid (ABA) from carotenoids via the mevalonic acid pathway to regulate plant development in response to salinity tolerance. Thus, the accumulation of carotenoids in the sprouts due to NaCl treatment may result from stimulation of the mevalonic acid pathway [119].

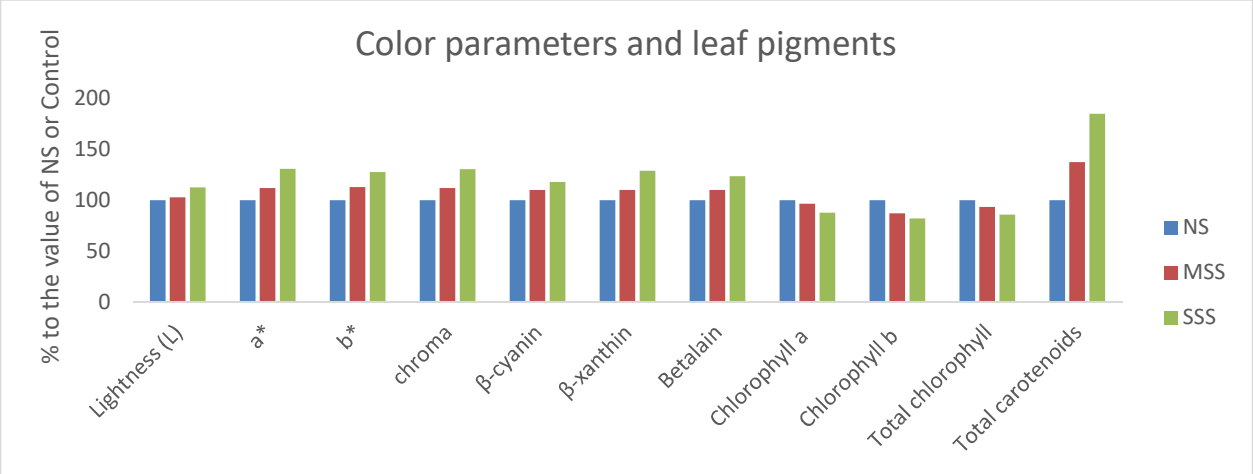

Fig. 1. Comparison of color parameters and leaf pigments (% to the value of NS or control) under three salinity levels: NS or Control (No saline water), MSS (Moderate salinity stress), SSS (Severe salinity stress) in three selected *A. tricolor* genotypes; a*, Redness/greenness; b*, Yellowness/blueness

Beta-carotene, ascorbic acid, TPC, TFC and TAC

Beta-carotene, ascorbic acid, total polyphenol content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) of *A. tricolor* were significantly affected by genotype, salinity level and the genotype × salinity stress interaction as presented in Fig. 2.

Within genotypes, the highest beta-carotene, ascorbic acid, TPC, TAC (DPPH) and TAC ($ABTS^+$) were observed in VA14, and the highest TFC was found in VA12 followed by VA14. Genotype VA12 exhibited the lowest beta-carotene, TPC, TAC (DPPH) and TAC ($ABTS^+$). Similarly, Alam *et al*. [118] reported variations in TPC, TFC, and TAC in different purslane accessions under salinity stress. Examination of the interaction of genotype × salinity stress indicated that VA14 under SSS exhibited the highest beta-carotene, ascorbic acid, TPC, TAC (DPPH) and TAC ($ABTS^+$), while VA12 under SSS had the highest TFC.

In contrast, the lowest beta-carotene, TPC, TAC (DPPH) and TAC ($ABTS^+$) was observed in VA12 under NS, while VA3 under NS showed the lowest ascorbic acid and TFC. In contrast, ascorbic acid was significantly increased with the increase in salinity stress in all the genotypes in the following order: NS < MSS < SSS. Higher beta-carotene was observed in

VA14 under MSS, VA12 under SSS, VA3 under SSS, and VA14 under NS, while a high ascorbic acid level was recorded in VA14 under MSS and VA14 under NS. A higher TPC was also found in VA3 under SSS and VA3 under MSS, while VA12 under MSS, VA14 under SSS and VA12 under NS had a high TFC. VA14 under MSS, VA3 under SSS, VA3 under MSS and VA3 under N showed a high TAC (DPPH), while VA14 under MSS, VA3 under SSS and VA12 under SSS had a high TAC ($ABTS^{+}$).

Fig. 2. Effect of genotype, salinity stress and genotype × salinity stress interaction on a) beta-carotene (mg g^{-1} FW), b) Ascorbic acid (mg 100 g^{-1} FW), c) Total polyphenol content (GAE µg g^{-1} dw), d) Total flavonoid content (RE µg g^{-1} dw), e) Total antioxidant capacity (DPPH) (TEAC µg g^{-1} dw) and (f) Total antioxidant capacity ($ABTS^{+}$) (TEAC µg g^{-1} dw) in three selected *A. tricolor* genotypes. Values are means of six replicates and different letters are differed significantly by Duncan Multiple Range Test ($P < 0.001$).

Petropoulos *et al.* [117] found an elevated response of phenolics, flavonoids and antioxidant activity with an increase in salt stress in *Cichorium spinosum*. Alam *et al.* [118] reported that different levels of salinity treatment resulted in 8–35% increases in the TPC, an approximately 35% increase in TFC, and 18–35% increases in FRAP activity in purslane. Lim

et al. [119] reported that buckwheat treated with 10, 50, and 100 mM after 7 d of cultivation were 57%, 121%, and 153% higher than that of the control, respectively. Ahmed *et al*. [247] reported an increase in phenolics and TAC (FRAP) with increasing NaCl concentrations in barley. In contrast, Neffati *et al*. [248] found a decrease in polyphenols and TAC (DPPH) with increasing NaCl concentrations in coriander. Salinity stress creates osmotic stress in plants by producing ROS that reduces the water potential and decreases stomatal conductivity, which restricts the CO_2 influx to leaves and results in an unfavorable CO_2/O_2 ratio in the chloroplasts, reducing photosynthesis. The plant can accumulate compatible solutes and antioxidants, such as ß-carotene, ascorbic acid, polyphenols, and flavonoids [255-257], that decrease the cytoplasmic osmotic potential, enabling water absorption to detoxify the ROS [265]. As a result, the plant can adapt to salinity stress and continue normal growth.

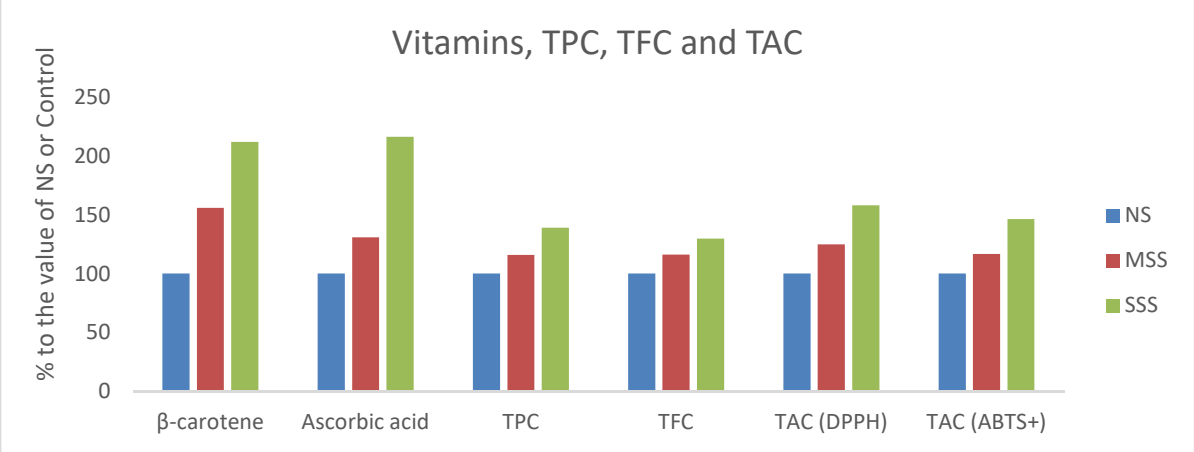

Fig. 3. Response of vitamins, TPC, TFC and TAC, (% to the value of NS or Control) under three salinity levels: NS or Control (No saline water), MSS (Moderate salinity stress), SSS (Severe salinity stress) in three selected *A. tricolor* genotypes; beta-carotene (mg g^{-1}), Ascorbic acid (mg 100 g^{-1}), TPC, Total polyphenol content (GAE µg g^{-1} dw); TFC. Total flavonoid content (RE µg g^{-1} dw); TAC (DPPH), Total antioxidant capacity (DPPH) (TEAC µg g^{-1} dw); TAC ($ABTS^{+}$), Total antioxidant capacity ($ABTS^{+}$) (TEAC µg g^{-1} dw)

Total biomass

The total biomass (g $plant^{-1}$) of *A. tricolor* was significantly affected by genotype, salinity level and the genotype × salinity stress interaction and is presented in Fig. 4.

Within genotypes, the highest biomass was observed in VA14 followed by the genotype VA12. Genotype VA3 exhibited the lowest biomass production. These results were fully consistent with those of Omami *et al*. [260] who reported that growth parameters decreased with increasing stress. They described that their sensitivity to salinity stress varied with the level of stress and genotype. In this study, total biomass was significantly and gradually

decreased with the increase in salinity stress in the following order: NS > MSS > SSS. In MSS and SSS, the total biomass decreased by 6.49% and 16.39% compared to the NS, respectively (Fig. 4). The highest total biomass was noted in NS, while the lowest total biomass was observed in SSS.

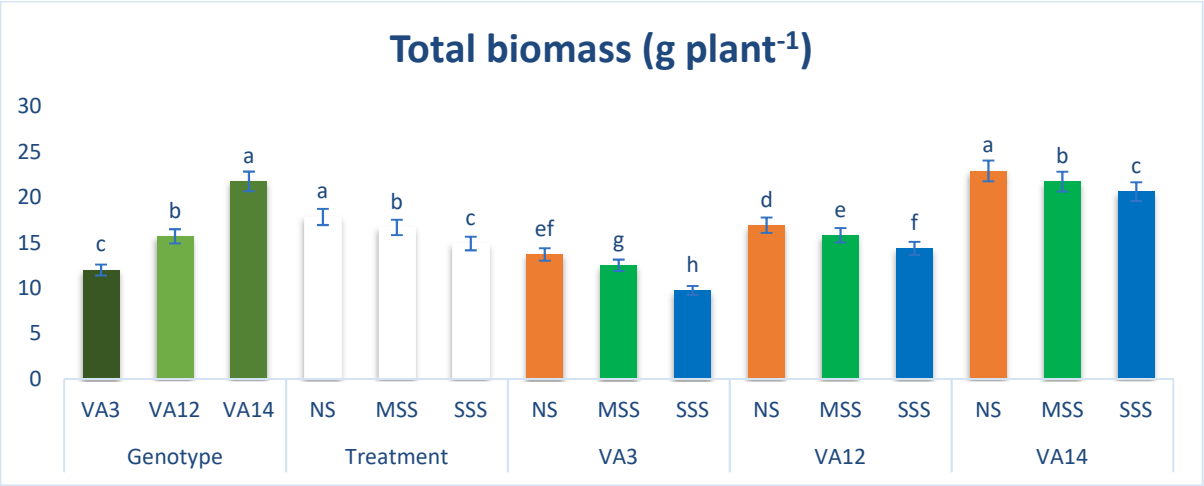

Fig. 4. Effect of genotype, salinity stress and genotype × salinity stress interactions on total biomass (g plant^{-1}) in three selected *A. tricolor* genotypes. NS or Control (No saline water), MSS (Moderate salinity stress), SSS (Severe salinity stress); values are means of six replicates and different letters are differed significantly by Duncan Multiple Range Test ($P < 0.01$).

The decrease in biomass production in *A. tricolor* was lower compared to the results of Menezes *et al*. [261] who observed that at 100 mM NaCl, the leaf dry mass, stem dry mass, root dry mass, total dry mass and leaf area of *A. cruentus* decreased by 73%, 74%, 49%, 70% and 74%, respectively, compared to the control. The *A. tricolor* studied is tolerant to salinity stress, which was consistent with the results of Omami [254], who reported that *A. tricolor* is a salt-tolerant genotype and can tolerate up to 200 mM NaCl.

The interaction of genotype × salinity stress indicated that VA14 under NS exhibited the highest total biomass, while VA3 under SSS had the lowest total biomass. All the genotypes showed a significant and gradual decline in total biomass with the increase in salinity stress in the following order: NS > MSS > SSS. However, VA3 had the highest decline in total biomass compared to other genotypes with the increase in the salinity stress in the following order: NS > MSS > SSS. In contrast, VA14 exhibited the lowest decline in total biomass compared to the other genotypes with the increase in salinity stress in the following order: NS > MSS > SSS, indicating more tolerance under salinity stress. Salinity stress caused reductions in biomass production in all the amaranth genotypes, although the relative effects varied, and the classification of the genotype for its salt tolerance would vary based on the biomass production. VA14 and VA12

exhibited more tolerance compared to VA3. The reduction in biomass production implies less assimilated production, therefore reducing the growth of the plants. The ability of a genotype that produces a large amount of biomass is important in characterizing genotypes as either salinity stress tolerant or susceptible. Genotypic differences in biomass production and partitioning under stress can be used as indicators of tolerance to salinity stress.

Correlation studies

The correlation coefficients of betacyanin, betaxanthin, betalain, chlorophyll a, chlorophyll b, total chlorophyll, total carotenoids, beta-carotene, ascorbic acid, TPC, TAC (DPPH) and TAC ($ABTS^+$) are presented in Table 3. Betacyanin, betaxanthin and betalain had highly significant correlations among each other, chlorophyll a, chlorophyll b, total chlorophyll, TPC, TAC (DPPH) and TAC ($ABTS^+$). Significant associations of these traits with TAC (DPPH) and TAC ($ABTS^+$) represented a crucial role of betacyanin, betaxanthin and betalain in the total antioxidant activity of *A. tricolor* leaves. Chlorophyll a, chlorophyll b, and total chlorophyll demonstrated significant associations among each other, betacyanin, betaxanthin and betalain, which indicated that the increase in any of the chlorophylls or betacyanin, betaxanthin or betalain simultaneously increased the rest of these five traits. Total carotenoids displayed significant relationships with beta-carotene, ascorbic acid, TFC, TAC (DPPH) and TAC ($ABTS^+$), demonstrating the vital role of carotenoid pigments in antioxidant activity. Beta-carotene showed highly significant interrelationships with ascorbic acid, TAC (DPPH), and TAC ($ABTS^+$), while this trait had significant associations with TPC and TFC. Similarly, ascorbic acid revealed significant interrelationships with TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$). Both beta-carotene and ascorbic acid played a vital role in the antioxidant activity of *A. tricolor*. TPC, TFC, and TAC (DPPH) were significantly interrelated with each other. The polyphenols and flavonoids of *A. tricolor* leaves established strong antioxidant activity. Alam *et al*. [122] reported a significant correlation of carotenoids, TPC, and TFC with TAC (FRAP) in salt-stressed purslane.

In conclusion, significant increases in L, a*, b*, chroma, betacyanin, betaxanthin, betalain, total carotenoids, beta-carotene, ascorbic acid, TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) were observed under salinity stress. All the antioxidant phytochemicals of *A. tricolor* leaves under MSS and SSS were very high compared to the corresponding control or NS values, which could be valuable food sources in modern diets and contribute considerably to human health. In addition, salt-stressed *A. tricolor* leaves had good sources of color parameters, total carotenoids, beta-carotene, ascorbic acid, TPC, TFC and unique sources of betacyanin and betaxanthin in leafy vegetables. The pigments, vitamins, phenolics and flavonoids showed

strong antioxidant activity due to their positive and significant interrelationships with TAC. Therefore, these pigments, vitamins, phenolics and flavonoids played a vital role in scavenging ROS and would benefit human health. In addition, *A. tricolor* cultivated under salinity stress could contribute to a high quality of the final product in terms of bioactive leaf pigments, vitamins and antioxidant profiles. It could be a promising alternative crop for farmers, especially in salinity prone areas and also coastal belts around the globe.

Table 3. Correlation co-efficient for antioxidant leaf pigments, vitamins, TPC, TFC and TAC in three selected *A. tricolor* genotypes

	betaxanthin (ng g^{-1})	Betalain (ng g-1)	Chl a (µg g^{-1})	Chl b (µg g^{-1})	T chl (µg g^{-1})	T car (mg 100 g^{-1})	β-carotene (mg g^{-1})	AsA (mg 100 g^{-1})	TPC (GAE µg g^{-1} dw	TFC (RE µg g^{-1} dw)	TAC (DPPH) (TEAC µg g^{-1} dw)	TAC ($ABTS^{+}$) (TEAC µg g^{-1} dw)
betacyanin	0.99**	0.98**	0.68*	0.79*	0.73*	0.24	0.49	0.17	0.87**	-0.61	0.85**	0.75*
betaxanthin		0.67*	0.65	0.77*	0.70*	0.21	0.49	0.16	0.88**	-0.57	0.87**	0.69*
Betalain			0.67*	0.78*	0.72*	0.21	0.50	0.16	0.88**	-0.56	0.86**	0.72*
Chl a				0.93**	0.99**	0.32	0.60	0.58	0.43	-0.67*	0.57	0.57
Chl b					0.97**	0.63	0.39	0.29	0.47	-0.30	0.56	0.26
T Chl						0.24	0.55	0.49	0.45	-0.05	0.58	0.37
T car							0.93**	0.90**	0.53	0.68*	0.69*	0.93**
beta-carotene								0.92**	0.68*	0.75*	0.82**	0.96**
AsA									0.72*	0.79*	0.76*	0.78*
TPC										0. 73*	0.95**	0.81**
TFC											0.84**	0.86**
TAC (DPPH)												0.99**

Chl a, Chlorophyll a; Chl b, Chlorophyll b; T chl, Total chlorophyll; T car, Total carotenoids; AsA, Ascorbic acid; TPC, Total polyphenol content (GAE µg g^{-1} dw); TFC, Total flavonoid content (RE µg g^{-1} dw); TAC (DPPH), Total antioxidant capacity (DPPH) (TEAC µg g^{-1} dw); TAC ($ABTS^{+}$), Total antioxidant capacity ($ABTS^{+}$) (TEAC µg g^{-1} dw); *significant at 5% level, ** significant at 1% level, (n = 6)

Abstract

A. tricolor is a unique source of betalain (betacyanin and betaxanthin) and a source of natural antioxidants, such as leaf pigments, vitamins, polyphenols, and flavonoids in leafy vegetables. It has substantial importance for food industry, since these compounds detoxify ROS in humans and are involved in defense against several diseases. In addition, previous research has shown that salt stress elevates these compounds in many leafy vegetables. Therefore, we evaluated the effect of salinity stress on these compounds. Three selected *A. tricolor* genotypes were studied under three salinity levels to evaluate the response of these compounds. Genotype, salinity stress and their interactions significantly affected all the traits studied. A significant and remarkable increase in L, a*, b*, chroma, betacyanin, betaxanthin, betalain, total carotenoids, beta-carotene, ascorbic acid, total polyphenolic content, total flavonoid content, and total antioxidant capacity were observed under 50 mM and 100 mM NaCl concentrations. Bioactive leaf pigments, beta-carotene, vitamin C, phenolics and flavonoids showed good antioxidant activity due to positive and significant interrelationships with total antioxidant capacity. *A.*

tricolor can tolerate salinity stress without compromising the high quality of the final product. Therefore, it could be a promising alternative crop in saline-prone areas around the globe.

3.3.3 Augmentation of leaf color parameters, pigments, vitamins, phenolic acids, flavonoids and antioxidant activity in selected A. tricolor under salinity stress

Purpose of the study

Salinity, one of the major abiotic stress and serious threat to global food security. It prohibits the cultivation of vegetables in many areas in the globe. It affects plants by creating nutritional imbalance, osmotic stress, water deficiency, and oxidative stress [192]. Moreover, previous studies demonstrated that high salinity changes the level of secondary metabolites in plants, including pigments, phenolic compounds and flavonoids, enhanced plant defense mechanisms against oxidative stress [262]. Salinity aggravates overproduction of reactive oxygen species (ROS) that results in oxidative damage by oxidizing proteins, lipids and DNA and other cellular macromolecules [88]. Plants have an excellent non-enzymatic network of ROS detoxification system through AsA, beta-carotene and carotenoids, phenolic compounds and flavonoids [88].

Amaranthus tricolor is an excellent source of leaf pigments, beta-carotene, vitamin C, phenolic acids, flavonoids and antioxidant capacity that had a great importance for the food industry as most of them are natural antioxidants and detoxify ROS in the human body [6, 35]. Hence, salt-stressed plants could economically be potential sources of antioxidants in the human life. These natural antioxidants play an important role in the human diet as involve in defense against several diseases like cancer, atherosclerosis, arthritis, cataracts, emphysema, and retinopathy, neuro-degenerative and cardiovascular diseases [8, 35, 48]. *A. tricolor* is a well-adapted leafy vegetable to different biotic and abiotic stresses and has multipurpose uses. Different factors such as biological, environmental, biochemical, physiological, ecological, and evolutionary processes are involved in the quantitative and qualitative improvement of natural antioxidants of this species of which, salinity stress can rapidly boost up the content of natural antioxidants [72]. There are few reports related to the effect of salinity stress on leaf pigments, vitamins, phenolic acids, flavonoids and antioxidant capacity in different crops including leafy vegetables.

Salt stress elevates vitamin C, phenolics, flavonoids and antioxidant activity in *Cichorium spinosum* [117]. Alam *et al*. [118] observed different levels of salinity treatment resulted in 8–35% increase in TPC; about 35% increase in TFC; and 18–35% increase in FRAP activity in purslane. Lim *et al.* [119] reported that buckwheat treated with 10, 50, 100, and 200 mM NaCl concentrations result in an increase of phenolic compounds and carotenoids in the sprouts compared to the control (0 mM). The buckwheat sprouts treated with 10, 50, and 100 mM NaCl after 7 d of cultivation were 57%, 121%, and 153%, higher phenolic content than

that of the control condition, respectively. In plants, polyphenol synthesis and accumulation are mostly stimulated in response to salinity [251]. Thus, salt-stressed plants might represent potential sources of polyphenols. To our knowledge, there is no information on *A. tricolor* in response to salinity stress in terms of leaf pigments, beta-carotene, vitamin C, phenolic acids, flavonoids and antioxidant capacity. In our previous studies, we selected some antioxidant enriched and high yield potential genotypes [143, 149-151, 160-162, 173]. Therefore, in present investigation, high antioxidant enriched and high yield potential genotype VA13 were evaluated to study the response of leaf pigments, beta-carotene, vitamin C, phenolic acids, flavonoids and antioxidant capacity under four salinity stress.

Materials and methods

Experimental site, Plant materials and experimental conditions

Earlier, we collected 102 genotypes in different eco-geographical regions of Bangladesh. On the basis of our previous studies [143, 149-151, 160-162, 173], an antioxidant enriched high yield potential genotype (Accession VA13) was selected for this investigation. This genotype was grown in pots of a rain shelter open field of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh (AEZ-28, $24^0 23'$ north latitude, $90^0 08'$ east longitude, 8.4 m.s.l.). The seeds were sown in plastic pots (15 cm in height and 40 cm length and 30 cm width) in a randomized complete block design (RCBD) with three replications. N: P2O5:K2O were applied @92:48:60 kg ha^{-1} as a split dose. First, in pot soil, @46:48:60 kg ha^{-1} N: P2O5:K2O and second, at 7 days after sowing (DAS) @46:0:0 kg ha^{-1} N: P2O5:K2O. The genotype was grouped into three sets and subjected to four salinity stress treatments that are, 100 mM NaCl, 50 mM NaCl, 25 mM NaCl, and control or no saline water (NS). Pots were well irrigated with fresh water every day up to 10 days after sowing (DAS) of seeds for proper establishment and vigorous growth of seedlings. Imposition of salinity stress treatment was started at 11 DAS and continued up to 40 DAS (edible stage). Saline water (100 mM NaCl, 50 mM NaCl and 25 mM NaCl) and fresh water were applied to respective pots once a day. At 40 DAS the leaves of *Amaranthus tricolor* were harvested. All the parameters were measured in six samples.

Chemicals

Solvent: methanol and acetone. Reagents: Standard compounds of pure phenolic acids, HPLC grade acetonitrile and acetic acid, vitamin C, gallic acid, rutin, methanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), $ABTS^+$ (2,2-azinobis-3-ethylenzothiazoline-6-sulphonic acid), trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), aluminum chloride hexahydrate,

sodium carbonate, potassium acetate, Folin-Ciocalteu reagent, Caesium chloride, dithiothreitol (DTT) and potassium persulfate. All solvents and reagents used in this study were of high purity laboratory products obtained from Kanto Chemical Co. Inc. (Tokyo, Japan) and Merck (Germany).

Leaf color measurement

Leaf color were measured following the procedure described in the previous chapter

Determination of betacyanin and betaxanthin content

Betacyanin and betaxanthin were extracted from fresh amaranth leaves following the procedure described in the previous chapter

Determination of total carotenoids

Total carotenoids were determined from 80% acetone extracts following the procedure described in the previous chapter

Beta-carotene

The extraction and estimation of beta-carotene were performed following the procedure described in the previous chapter

Vitamin C

Vitamin C was measured following the procedure described in the previous chapter

Extraction of samples for TPC, TFC and TAC analysis

Samples were extracted following the procedure described in the previous chapter

Determination of TPC, TFC and TAC

TPC, TFC and TAC were measured following the procedure described in the previous chapter

Extraction of samples for HPLC and LC-MS analysis

Samples were extracted following the procedure described in the previous chapter

HPLC analysis of phenolic acids and flavonoids

Phenolic acids and flavonoids were measured following the procedure described in the previous chapter

Statistical Analysis

The data was statistically analyzed by analysis of variance (ANOVA) using Statistix 8 software and the means were compared by Duncan's Multiple Range Test (DMRT) at 1% level of probability. The results were reported as the mean ± SD of three separate replications.

Results and Discussion

Effect of salinity on leaf color parameters and leaf pigments

Leaf color parameters and leaf pigments under different salinity stress are presented in Table 1. Leaf color is one of the most important parameters for consumers, playing a crucial role in choice making, preference and acceptability of the product, and may also be considered as an indicator for estimating the antioxidant properties of the leafy vegetables [259]. High redness and yellowness values recorded in the genotype VA13 could be expected since it is characterized by the presence of the high pigments (anthocyanins, carotenoids, betacyanin, betaxanthin and betalain). The results obtained in the present study were fully agreed with the results of Colonna *et al*. [259]. L*, a*, b*, chroma, betacyanin, betaxanthin, betalain, and total carotenoids were remarkably increased with the severity of salinity stress in the order, Control (No saline water) < Low salinity stress (LSS) < Moderate salinity stress (MSS) < Severe salinity stress (SSS). At LSS, MSS and SSS conditions, L*, a*, b*, chroma, betacyanin, betaxanthin, betalain and total carotenoids were increased by (4%, 6%, 5%, 3%, 1% 2%, 0.91% & 2%), (10%, 13%, 11%, 9%, 5% 7%, 5% & 24%) and (13%, 25%, 17%, 17%, 9% 12%, 8% & 50%), respectively compared to control condition (Fig. 1). Lim *et al*. [119] observed continuous increment in the level of carotenoids in response to all NaCl concentrations tested. They reported the greatest difference between the carotenoid content with 50 or 100 mM NaCl which was higher double than that of control sprouts, while treatment with 10 or 200 mM NaCl resulted 40% increase in carotenoids. Unlike other biotic and abiotic stresses, salinity stress induces biosynthesis of abscisic acid (ABA) from carotenoids via mevalonic acid pathway in order to regulate plant development in response to salinity tolerance. Thus, due to NaCl treatment, accumulation of carotenoids in the sprouts might be due to stimulation of the mevalonic acid pathway [119]. Alam *et al*. [118] reported both increment and decrement in total carotenoid contents in different accessions of purslane with the severity of salinity stress.

Table 1. Effect of salinity on leaf color parameters and leaf pigments in selected *A. tricolor* genotype

Salinity stress	Color parameters				Antioxidant leaf pigments			
	L*	a*	b*	Chroma	betacyanin (ng g^{-1})	betaxanthin (ng g^{-1})	Betalain (ng g-1)	Total carotenoids (mg 100 g^{-1})
Control (No saline water)	31.16 ± 1.85a	10.12 ± 0.87a	3.56 ± 0.28a	12.46 ± 0.52a	624.75 ± 2.54a	266.44 ± 2.81a	902.62 ± 4.52a	35.75 ± 1.24a
Low salinity stress (LSS)	32.34 ± 1.92b	10.76 ± 0.99b	3.75 ± 0.32b	12.88 ± 0.67b	632.83 ± 3.08b	273.72 ± 3.24b	910.87 ± 4.22b	36.52 ± 1.35b
Moderate salinity stress (MSS)	34.12 ± 2.05c	11.42 ± 1.12c	3.96 ± 0.24c	13.62 ± 0.46c	654.62 ± 3.28c	285.68 ± 4.02c	945.56 ± 3.57c	44.68 ± 1.57c
Severe salinity stress (SSS)	35.16 ± 2.14d	12.63 ± 1.02d	4.16 ± 0.22d	14.54 ± 0.44d	678.92 ± 2.98d	298.84± 3.87d	978.42 ± 3.92d	53.87 ± 0.98d

Different letters in a column are differed significantly by Duncan Multiple Range Test ($P < 0.01$)

Impact of salinity on beta-carotene, vitamin C, TPC, TFC and TAC

Beta-carotene, vitamin C, total polyphenol content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) of the genotype of *A. tricolor* were significantly affected by

salinity levels (Fig. 2). The significant increase in beta-carotene, vitamin C, TPC, TFC, TAC (DPPH) and TAC ($ABTS^{+}$) due to salinity stress were found in the order: Control < LSS < MSS < SSS. At LSS, MSS and SWS conditions, beta-carotene, vitamin C, TPC, TFC, TAC (DPPH) and TAC ($ABTS^{+}$) were increased by (8%, 13%, 4%, 5%, 5% and 8%), (43%, 66%, 20%, 17%, 28% and 30%) and (101%, 192%, 36%, 23%, 52% and 59%), compared to control condition, respectively (Fig. 3). beta-carotene, vitamin C, TPC, TFC, TAC (DPPH) and TAC ($ABTS^{+}$) had the highest values under SSS condition, while beta-carotene, vitamin C, TPC, TFC, TAC (DPPH) and TAC ($ABTS^{+}$) were observed the lowest in control condition. Petropoulos *et al*. [117] found the elevated response of phenolics, flavonoids and antioxidant activity with the increase in salt stress in *Cichorium spinosum*. Alam *et al*. [118] reported that different levels of salinity treatment resulted 8–35% increases in TPC; about 35% increase in TFC; and 18–35% increases in FRAP activity in purslane. Lim *et al*. [119] reported that buckwheat treated with 10, 50, and 100 mM after 7 d of cultivation were 57%, 121%, and 153%, higher phenolic content than that of the control, respectively. Ahmed *et al.* [247] reported increment in phenolics and TAC (FRAP) with increasing NaCl concentrations in barley. In contrast, Neffati *et al*. [248] found decrement in polyphenols and TAC (DPPH) with increasing NaCl concentrations in coriander.

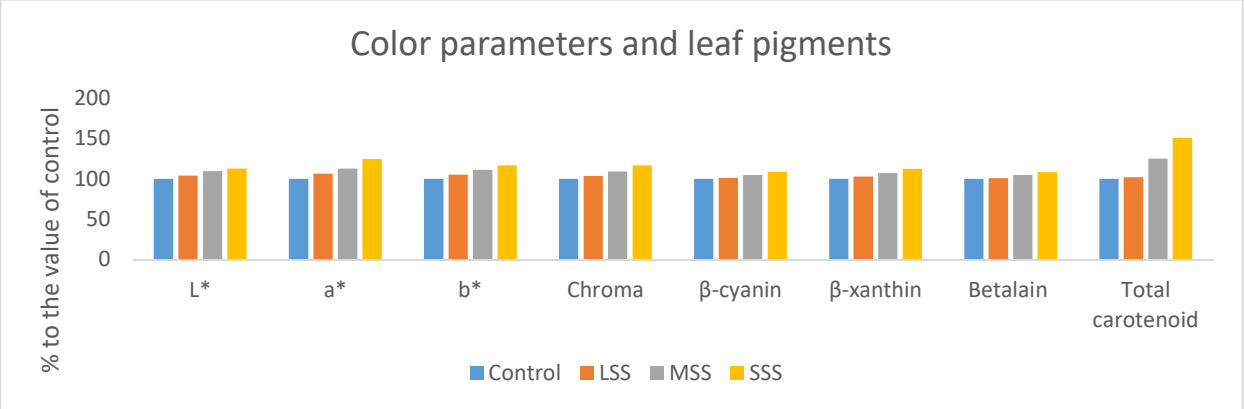

Fig. 1. Comparison of color parameters and leaf pigments (% to the value of control) under four salinity levels: Control (No saline water), LSS (Low salinity stress), MSS (Moderate salinity stress) and SSS (Severe salinity stress) in selected *A. tricolor* genotype; L*, Lightness; a*, Redness/greenness; b*, Yellowness/blueness

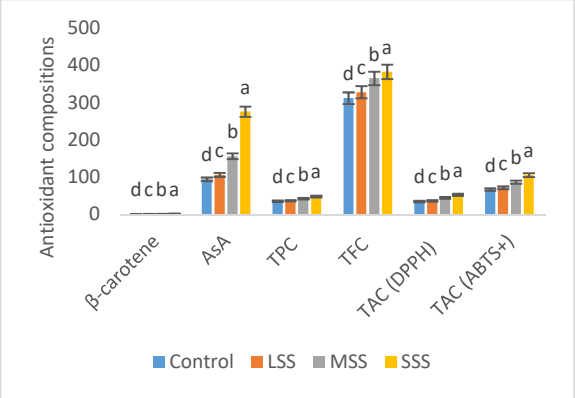

Fig. 2. Response to beta-carotene, Vitamin C, TPC, TFC and TAC under four salinity levels: Control (No saline water), LSS (Low salinity stress), MSS (Moderate salinity stress), SSS (Severe salinity stress) in selected *A. tricolor* genotype; beta-carotene (mg g^{-1}), AsA, Vitamin C (mg 100 g^{-1}); TPC, Total polyphenol content (GAE µg g^{-1} dw); TFC, Total flavonoid content (RE µg g^{-1} dw); TAC (DPPH), Total antioxidant capacity (DPPH) (TEAC µg g^{-1} dw); TAC ($ABTS^{+}$), Total antioxidant capacity ($ABTS^{+}$) (TEAC µg g^{-1} dw); (n = 6), different letters are differed significantly by Duncan Multiple Range Test ($P < 0.01$)

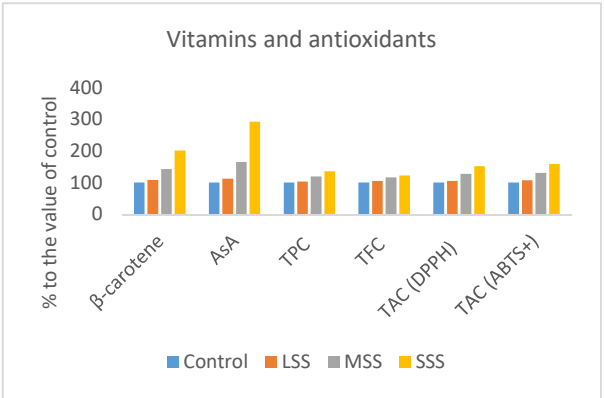

Fig. 3. Response to vitamins, TPC, TFC and TAC, (% to the value of control) under four salinity levels: Control (No saline water), LSS (Low salinity stress), MSS (Moderate salinity stress) and SSS (Severe salinity stress) in selected *A. tricolor* genotype; beta-carotene (mg g^{-1}), AsA, Vitamin C (mg 100 g^{-1}); TPC, Total polyphenol content (GAE µg g^{-1} dw); TFC. Total flavonoid content (RE µg g^{-1} dw); TAC (DPPH), Total antioxidant capacity (DPPH) (TEAC µg g^{-1} dw); TAC ($ABTS^{+}$), Total antioxidant capacity ($ABTS^{+}$) (TEAC µg g^{-1} dw)

Influence of salinity on phenolic acids and flavonoids

Data on retention time, λmax, molecular ion, main fragment ions in MS^2 and tentative compound identification for phenolic compounds are presented in Table 2. The values of phenolic acids and flavonoids components separated though LC from the genotype VA13 was compared with ion masses of standard phenolic acids and flavonoids by observing the particular peaks of the corresponding components. Totally, sixteen phenolic compounds were identified including six hydroxybenzoic acids, seven hydroxycinnamic acids and three flavonoids. In this study, *trans*-cinnamic acid was newly identified phenolic acid in *A. tricolor*. Except for *trans*-cinnamic acid, Khanam and Oba [179] in red and green amaranths, Khanam *et al*. [174] in eight different leafy vegetables including amaranths described the rest 15 phenolic acids and flavonoids with normal cultivation practices. However, an attempt was made for the first time to evaluate the effect of sixteen phenolic acids and flavonoids of *A. tricolor* under four salinity stress. Quantification of identified phenolic compounds in selected *Amaranthus tricolor* leaves under four salinity stress are presented in Table 3. Considering phenolic acids and flavonoids, hydroxybenzoic acids having one functional carboxylic acid were the most plentiful compounds in this genotype. Within hydroxybenzoic acids, salicylic acid was found to be as one of the main phenolic acids followed by vanilic acid and gallic acid. Gallic acid and *p*- acid was the most abundant compound followed by *trans*-cinnamic acid and *m*-coumaric acid. hydroxybenzoic acid content of the genotype VA13 under control condition were higher than *A. tricolor* genotypes of Khanam *et al*. [174]. Regarding hydroxycinnamic acids, chlorogenic

A good amount of caffeic acid, *p*-coumaric acid, ferulic acid were also observed in this genotype. The genotype VA13 had higher caffeic acid and *m*-coumaric acid under control condition compared to *A. tricolor* genotypes of Khanam *et al*. [174]. The hydroxycinnamic acids synthesized from phenylalanine are the most extensively disseminated phenolic acids in plant tissues [180]. In plants, flavonoids occasionally occur as a glycone, although the most common forms are glycoside derivatives. These compounds account for 60% of total dietary phenolic compounds [181, 263]. Flavonols are the most prevalent flavonoids in the plant kingdom and glycosides of quercetin are the most predominant naturally occurring flavonols [181]. In this investigation, the flavonoids, rutin (quercetin-3-rutinoside) and isoquercetin

Table 2. Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data and tentative identification of phenolic compounds in selected *Amaranthus tricolor* leaves.

Peak no	Rt (min)	λ_{max} (nm)	Molecular ion $[M - H]^-$ (m/z)	MS^2 (m/z)	Identity of tentative compounds
1	9.1	254	169	169.2	3,4-5 Trihydroxybenzoic acid
2	30.6	254	167	167.2	4-hydroxy-3-methoxybenzoic acid
3	34.8	254	197	197.1	4-Hydroxy-3,5-dimethoxybenzoic acid
4	31.5	254	137	137.2	4-hydroxybenzoic acid
5	48.2	254	137	137.2	2-Hydroxybenzoic acid
6	52.5	254	301	301.1	(2,3,7,8-tetrahydroxy-chromeno [5,4,3-cde]chromene-5,10-dione
7	32.0	280	179	179.1	3,4-Dihydroxy-trans-cinnamate
8	31.1	280	353	353.2	3-(3,4-Dihydroxycinnamoyl) quinic acid
9	42.0	280	163	163.1	4-hydroxycinnamic acid
10	47.9	280	193	193.2	4-hydroxy-3-methoxycinnamic acid
11	49.6	280	163	163.3	3-hydroxycinnamic acid
12	49.0	280	223	223.2	4-Hydroxy-3,5-dimethoxycinnamic acid
13	67.3	280	147	147.1	3-Phenylacrylic acid
14	54.3	360	463	463.3	Quercetin-3-glucoside
15	53.3	360	463	463.5	Quercetin-3-galactoside
16	53.0	360	609	609.4	Quercetin-3-rutinoside

Table 3. Quantification of identified phenolic compounds (µg g^{-1} FW) in selected *Amaranthus tricolor* leaves under four salinity stress.

Phenolic group	Compound	Control (No NaCl)	LSS (25 mM NaCl)	MSS (50 mM NaCl)	SSS (100 mM NaCl)
Hydroxybenzoic acid					
Gallic acid	3,4-5 Trihydroxybenzoic acid	6.64 ± 0.05c	6.67 ± 0.06c	8.46 ± 0.06b	9.39 ± 0.08a
Vanilic acid	4-hydroxy-3-methoxybenzoic acid	9.40 ± 0.12c	9.37 ± 0.09c	12.65 ± 0.08b	14.89 ± 0.22a
Syringic acid	4-Hydroxy-3,5-dimethoxybenzoic acid	1.46 ± 0.01b	1.26 ± 0.02d	1.43 ± 0.01c	1.52 ± 0.02a
p-hydroxybenzoic acid	4-hydroxybenzoic acid	2.75 ± 0.02c	2.76 ± 0.03c	3.87 ± 0.02b	4.24 ± 0.01a
Salicylic acid	2-Hydroxybenzoic acid	16.53 ± 0.42d	17.85 ±0.24c	24.87 ± 0.35b	28.61 ± 0.61a
Ellagic acid	(2,3,7,8-tetrahydroxy-chromeno [5,4,3-cde]chromene-5,10-dione	1.16 ± 0.03c	1.23 ± 0.05b	2.36 ± 0.06a	2.38 ± 0.03a
Total benzoic acids		37.95	39.14	53.63	61.03
Hydroxycinnamic acid					
Caffeic acid	3,4-Dihydroxy-trans-cinnamate	1.46 ± 0.03c	1.45 ± 0.02c	1.83 ± 0.04b	2.58 ± 0.06a
Chlorogenic acid	3-(3,4-Dihydroxycinnamoyl) quinic acid	7.38 ± 0.32d	7.98 ± 0.52c	9.82 ± 0.28b	12.65 ± 0.48a
p-coumaric acid	4-hydroxycinnamic acid	1.16 ± 0.01d	1.25 ± 0.01c	2.53 ± 0.02b	2.62 ± 0.03a
Ferulic acid	4-hydroxy-3-methoxycinnamic acid	1.20 ± 0.02c	1.16 ± 0.02c	2.05 ± 0.04b	3.19 ± 0.05a
m-coumaric acid	3-hydroxycinnamic acid	2.87 ± 0.05c	2.87 ± 0.06c	5.25 ± 0.04b	7.36 ± 0.03a
Sinapic acid	4-Hydroxy-3,5-dimethoxycinnamic acid	0.35 ± 0.01b	0.36 ± 0.01b	0.43 ± 0.01a	0.45 ± 0.01a
Trans-cinnamic acid	3-Phenylacrylic acid	6.85 ± 0.02b	6.86 ± 0.01b	6.89 ± 0.02a	6.92 ± 0.03a
Total cinnamic acids		21.28	21.93	28.80	35.77
Flavonoids					
Iso-quercetin	Quercetin-3-glucoside	4.66 ± 0.21c	4.80 ± 0.24c	7.23 ± 0.16b	9.24 ± 0.18a
Hyperoside	Quercetin-3-galactoside	1.35 ± 0.02b	1.33 ± 0.01b	2.43 ± 0.01a	2.44 ± 0.02a
Rutin	Quercetin-3-rutinoside	6.62 ± 0.11d	6.74 ± 0.09c	8.87 ± 0.08b	9.92 ± 0.14a
Total flavonoids		**12.63**	**12.87**	**18.53**	**21.60**
Total phenolic acids		**59.23**	**61.07**	**81.43**	**96.80**
Total phenolic index		**71.86**	**73.94**	**100.96**	**118.40**

Different letters in a row are differed significantly by Duncan Multiple Range Test ($P < 0.01$); ($n = 6$)

(quercetin-3-glucoside) were the most abundant in this genotype. The genotype VA13 exhibited higher rutin (quercetin-3-rutinoside) content under control condition in comparison to *A. tricolor* genotypes of Khanam *et al*. [174]. Three hydroxybenzoic acids (Gallic acid, vanilic acid and *p*-hydroxybenzoic acid); three hydroxycinnamic acid (Caffeic acid, ferulic acid and *m*-coumaric acid) and flavonoids iso-quercetin had no significant differences in their composition between control and LSS, however, the compositions of these acids were increased significantly from MSS to SSS. In MSS and SSS, the composition of these phenolic acids and flavonoids were increased by (27%, 35%, 41%, 25%, 71% 83% and 55%) and (41%, 58%, 54%, 77%, 166% 156% and 98%); respectively (Fig. 4 and 5). Salicylic acid, chlorogenic acid, *p*-coumaric acid and rutin were remarkably increased with the severity of salinity stress (Control < LSS < MSS < SSS). In LSS, MSS and SSS, the concentration of these phenolic acids and flavonoids were increased by (8%, 8%, 8% and 2%); (50%, 33%, 18% and 34%) and (73%, 71%, 26% and 50%); respectively (Fig. 4 and 5). Sinapic acid, *trans*-cinnamic acid, and hyperoside had no significant differences in their composition at control and LSS condition, however, the compositions of these acids were increased significantly under MSS or SSS condition compared to control and LSS condition. The composition of these acids under MSS or SSS was statistically similar. The ellagic acid content was significantly increased in the order: Control < LSS < MSS = SSS by 6% and 103% at LSS and MSS or SSS, respectively (Fig. 4 and 5); while syringic acid concentration was increased in the order: LSS < MSS < Control < SSS. Except for syringic acid, all the phenolic acids and flavonoids exhibited low concentrations under control condition, whereas these acids had the highest concentrations under SSS condition. Lim *et al*. [119] reported that buckwheat sprouts treated with 10, 50, and 100 mM NaCl after 7 d of cultivation were 57%, 121%, and 153%, higher phenolic content

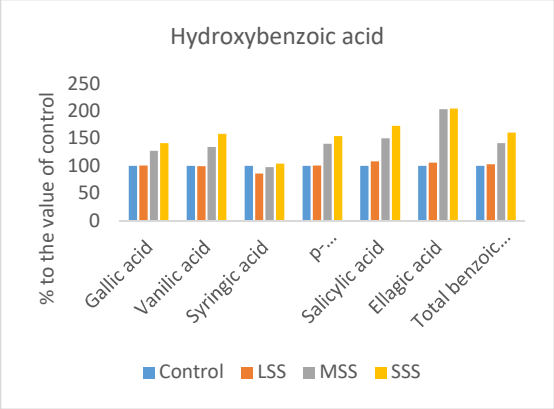

Fig. 4. Changes of hydroxybenzoic acid compositions (µg g^{-1} FW) (% to the value of control) under four salinity levels: Control (No saline water), LSS (Low salinity stress), MSS (Moderate salinity stress) and SSS (Severe salinity stress) in selected *A. tricolor* genotype

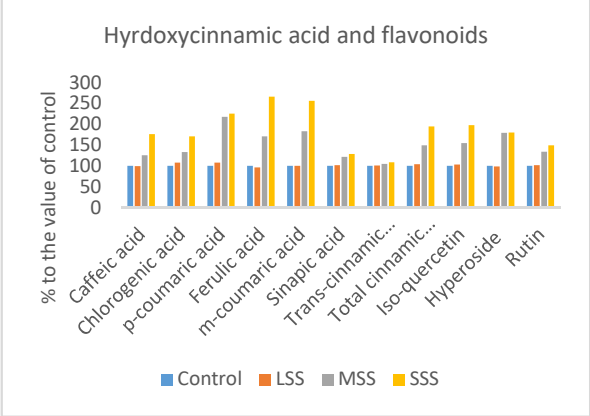

Fig. 5. Changes of hydroxycinnamic acid and flavonoid compositions (µg g^{-1} FW) (% to the value of control) under four salinity levels: Control (No saline water), LSS (Low salinity stress), MSS (Moderate salinity stress) and SSS (Severe salinity stress) in selected *A. tricolor* genotype

than that of the control condition, respectively. The total phenolic compounds ranged from 65.86 to 112.40 µg g^{-1} extract, with a significant and sharp increment from control to SSS in the following order: Control < LSS < MSS < SSS. Klados and Tzortzakis [264] reported a significant increase in total phenolic acids and flavonoids content with increasing salinity in *Cichorium spinosum*. Similarly, total phenolic acids and total flavonoids ranged from 53.23 to 90.80 and 12.63 to 21.60 µg g^{-1} extract, respectively with significantly and sharply increased from control to SSS (Control < LSS < MSS < SSS). Petropoulos *et al*. [117] found elevated response of phenolic acids and flavonoids with the increase in salt stress in *Cichorium spinosum*. Ahmed *et al*. [247] reported increment of phenolic acids with increasing NaCl concentrations in barley.

Correlation studies

The correlation coefficient among betacyanin, betaxanthin, betalain, total carotenoids, beta-carotene, ascorbic acid, TPC, TAC (DPPH) and TAC ($ABTS^+$) are presented in Table 4. betacyanin, betaxanthin and betalain had highly significant positive correlations among each other and with TPC, TAC (DPPH) and TAC ($ABTS^+$). Significant association between TAC (DPPH) and TAC ($ABTS^+$) represented a crucial role of betacyanin, betaxanthin and betalain in the total antioxidant activity of *A. tricolor* leaves. Total carotenoids displayed significant relationships with beta-carotene, vitamin C, TFC, TAC (DPPH) and TAC ($ABTS^+$) demonstrating the vital role of carotenoid pigments in the antioxidant activity. Beta-carotene showed highly significant interrelationships with vitamin C, TAC (DPPH) and TAC ($ABTS^+$) and significant association with TPC and TFC. It indicated that increase in beta-carotene was directly related to the increment of TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$). Similarly,

Table 4. Correlation coefficient for antioxidant leaf pigments, vitamins, TPC, TFC and TAC in selected *A. tricolor* genotype

	betaxanthin (ng g^{-1})	Betalain (ng g-1)	Total carotenoids (mg 100 g^{-1})	beta-carotene (mg g^{-1})	Vitamin C (mg 100 g^{-1})	TPC (GAE µg g^{-1} dw	TFC (RE µg g^{-1} dw)	TAC (DPPH) (TEAC µg g^{-1} dw)	TAC ($ABTS^+$) (TEAC µg g^{-1} dw)
betacyanin	0.96**	0.95**	0.32	0.37	0.18	0.87**	-0.65	0.87**	0.75*
betaxanthin		0.76*	0.24	0.42	0.14	0.88**	-0.47	0.82**	0.77*
Betalain			0.29	0.48	0.12	0.88**	-0.49	0.88**	0.82*
T carotenoids				0.92**	0.95**	0.53	0.67*	0.74*	0.96**
beta-carotene					0.98**	0.68*	0.72*	0.83**	0.92**
Vitamin C						0.32	0.35	0.82*	0.88*
TPC							0. 78*	0.98**	0.84**
TFC								0.87**	0.89**
TAC (DPPH)									0.97**

T carotenoids, Total carotenoids; TPC, Total polyphenol content (GAE µg g^{-1} dw); TFC, Total flavonoid content (RE µg g^{-1} dw); TAC (DPPH), Total antioxidant capacity (DPPH) (TEAC µg g^{-1} dw); TAC ($ABTS^+$), Total antioxidant capacity ($ABTS^+$) (TEAC µg g^{-1} dw); *significant at 5% level, ** significant at 1% level, (n = 6)

vitamin C revealed significant interrelationship with TAC (DPPH) and TAC ($ABTS^+$). Both beta-carotene and vitamin C played a vital role in the antioxidant activity of *A. tricolor*. In

contrast, vitamin C exerted negligible insignificant association with TPC and TFC. Jimenez-Aguilar and Grusak [178] found similar results for vitamin C in different species of *Amaranthus*. TPC, TFC and TAC (DPPH) were found significantly interrelated among each other. Alam *et al.* [118] also reported significant correlation of carotenoids, TPC, TFC with TAC (FRAP) in salt-stressed purslane. Significant positive interrelationship of TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) signify that TPC, TFC had strong antioxidant activity. Similarly, significant positive association between TAC (DPPH) and TAC ($ABTS^+$) confirmed the validation of antioxidant capacity of *A. tricolor* by two different methods of antioxidant capacity measurement. Leaf pigments, beta-carotene, vitamin C, TPC and TFC had strong antioxidant activity as these bioactive compounds showed significant association with TAC (DPPH) and TAC ($ABTS^+$).

In conclusion, at MSS and SSS conditions, leaf color parameters and pigments, vitamins, phenolic acids, flavonoids and antioxidant capacity of *A. tricolor* leaves were very high compared to control condition. Hence, salt-stressed *A. tricolor* leaves had a good source of natural antioxidants compared to plant grown in normal cultivation practices. The correlation coefficient revealed strong antioxidant activity of leaf pigments, beta-carotene, vitamin C, TPC, TFC that could be contributed as a valuable food source for human diets and health benefit. *A. tricolor* cultivated under salinity stress could be contributed as a high-quality product in terms of leaf pigments, bioactive compounds, vitamins, phenolic acids, flavonoids and antioxidants. It can be a promising alternative crop for farmers, especially in salt affected areas and also coastal belt in the world.

Abstract

A. tricolor genotype VA13 was evaluated under four salinity stress in terms of color parameters, leaf pigments, beta-carotene, vitamin C, TPC, TFC, TAC, phenolic acids and flavonoids. Salinity stress significantly increases all the studied traits. The increments of all these compounds were high under moderate and severe salinity stress compared to control condition. In this study, *trans*-cinnamic acid was newly identified phenoic acid in *A. tricolor*. Salicylic acid, vanilic acid, *trans*-cinnamic acid, gallic acid, chlorogenic acid, rutin, isoquercetin and *m*-coumaric acid were the most abundant phenolic compounds of amaranth that increased with the severity of salinity stress. *A. tricolor* leaves are good source of pigments, beta-carotene, vitamin C, bioactive compounds, phenolic acids, flavonoids and antioxidants. In salt-stressed amaranth, correlation studies revealed strong antioxidant activity of leaf pigments, beta-carotene, vitamin C, TPC, TFC. These bioactive compounds played a vital role in scavenging

ROS and could be beneficial to human nutrition by serving as a good antioxidant and antiaging source in human health benefit. *A. tricolor* cultivated under salinity stress conditions can contribute a high quality of the final product in terms of leaf pigments, bioactive compounds, phenolic acids, flavonoids and antioxidants. It can be a promising alternative crop in saline-prone areas.

CHAPTER 4

GENERAL DISCUSSION

Anemia, night blindness, scurvy is the problem for poor child community in the third world countries including Indian subcontinent. Iron, beta carotene and ascorbic acid are also important for recovery of anemia, night blindness and scurvy, respectively. Antioxidant vitamins and minerals are important constituents of the human diet by serving as cofactors for many physiological and metabolic processes.

Variability plays a vital role in the selection of superior genotypes in crop improvement programs. Pronounced variation in the breeding materials is a prerequisite for development of varieties to fulfill the existing demand. Creation of variability is prerequisite for crop breeders. Morphological and agronomic traits are quantitative in nature, and interact with the environment under study, so partitioning the traits into genotypic, phenotypic, and environmental effects is essential to find out the additive or heritable portion of variability. In the present investigation, the range of variation was much pronounced for all the traits except Ca, Mg, K, protein and beta-carotene content indicating a wide range of variability among the genotypes studied. High genotypic and phenotypic variances were observed for Fe, Zn, Mn, ascorbic acid, plant height, leaves per plant, leaf area, shoot/root weight, shoot weight, dietary fiber content and biological yield indicating the presence of the wide range of variability among the traits in vegetable amaranth. In contrast, Ca, Mg, K, protein and beta-carotene content showed low genotypic and phenotypic variances that indicated no scope of selection on the basis of these traits for improvement of vegetable amaranth crop. Fe, Zn, Mn, ascorbic acid, plant height, leaves per plant, leaf area, shoot/root weight, shoot weight, dietary fiber content foliage yield and biological yield had close differences in genotypic and phenotypic variances along with genotypic coefficient of variability (GCV) and phenotypic coefficient of variability (PCV) values, which indicated preponderance of additive gene effects for these traits i. e., less environmental influence in the expression of these traits or the major portion of the phenotypic variance was genetic in nature and greater scope of improvement of vegetable amaranth through selection. Variability alone is not of much help in determining the heritable portion of variation. The amount of gain expected from a selection depends on heritability and genetic advance in a trait. Heritability has been widely used to assess the degree to which a character may be transmitted from parent to offspring. Knowledge of heritability of a character is important as it indicates the possibility and extent to which improvement is possible through

selection [135]. However, high heritability alone is not enough to make sufficient improvement through selection generally in advance generations unless accompanied by a substantial amount of genetic advance [136]. The expected genetic advance is a function of selection intensity, phenotypic variance, and heritability and measures the differences between the mean genotypic values of the original population from which the progeny is selected. It has been emphasized that genetic gain should be considered along with heritability in coherent selection breeding program [19]. It is considered that if a trait is governed by non-additive gene action it may give high heritability but low genetic advance, which limits the scope for improvement through selection, whereas if it is governed by additive gene action, heritability and genetic advance would be high, consequently substantial gain can be achieved through selection. In these studies, the heritability was high for all the traits except beta carotene indicated the preponderance of additive gene action for these traits. High heritability coupled with high GA in percent of mean was observed for all the traits except Mg indicated that were govern to a great extent by additive gene. So, selection based on these traits would be effective for the improvement of vegetable amaranth.

The genotypic correlation showed some interesting results. In the present investigation, the genotypic correlation coefficients were very much close to the corresponding phenotypic values for all the traits indicating additive type of gene action i.e., less environmental influence on the expression of the traits. The higher magnitude of genotypic correlation than respective phenotypic correlations between various characters in amaranth have also been reported by Shukla *et al*. [23] and Shukla and Singh [18]. It was revealed that foliage yield had a significant positive correlation with iron, manganese, protein, fiber content, ascorbic acid, plant height, leaves per plant and stem base diameter indicating selection for high iron, manganese, protein, fiber, ascorbic acid content and tall and thick plant with more leaves were closely associated with high foliage yield i.e., increase in with iron, manganese, protein, fiber content, ascorbic acid, plant height, leaves per plant and stem base diameter could lead to increase the foliage yield of vegetable amaranth genotypes. Shukla *et al.* [23] observed positive association of foliage yield with beta-carotene and ascorbic acid, plant height, diameter of stem base and fiber content [23]. Similarly, Sarker and Mian [137] observed significant positive association between yield and its contributing traits in rice. Biological yield had significant positive correlation with leaf area (0.326), shoot weight (0.999), shoot/root weight (0.454) and stem base diameter (0.368), indicating that biological yield of vegetable amaranth could be increased with the increase of leaf area, shoot weight, shoot/root weight and stem base diameter. Sarker *et al*. [143] observed that foliage yield was highly associated with plant height, leaf area,

leaves/plant stem base diameter and dietary fiber content. Plant height had significant exhibited significant positive association with leaves per plant and stem base diameter. A Similar trend was observed by earlier work in *A. tricolor* [23]. Insignificant genotypic correlation was observed among nutrient, antioxidant, yield and yield contributing morphological, quality, agronomic traits and biological yield, except K *vs.* Mg (0.753), protein *vs.* dietary fiber (–0.295), and stem base diameter *vs.* Ca (–0.491). This indicates that selection for high mineral, vitamins, protein and dietary fiber content, nutrient and antioxidant might be possible without compromising yield loss. On the other hand, most of the interrelationships among different agronomic traits were significant. Stem base diameter had a significant positive association with leaf area (0.597), and shoot weight (0.365), whereas these traits showed significant negative association with Ca (-0.491). Shoot/root weight exhibited significant positive interrelationship with shoot weight (0.454) indicating that plant with thick stem contained less Ca, more leaves and shoot weight. Significant positive association was observed between shoot weight and leaf area (0.326).

Considering high genotypic and phenotypic variances along with genotypic coefficient of variability and phenotypic coefficient of variability values, high heritability coupled with high genetic advance and genetic advance in percent of mean, Fe, Mn, Zn, protein, beta-carotene, ascorbic acid, plant height, leaves per plant, stem base diameter, leaf area, shoot/root weight, shoot weight, dietary fiber content, foliage yield and biological yield would be selected for the improvement of vegetable amaranth genotypes under study. However, correlation study revealed that strong positive association of Fe, Mn, protein, fiber, beta-carotene, ascorbic acid, plant height, leaves per plant, leaf area, shoot weight, shoot/root weight and stem base diameter with foliage yield. Selection based on Fe, Mn, protein, fiber, beta-carotene, ascorbic acid, plant height, leaves per plant, leaf area, shoot weight, shoot/root weight and stem base diameter could lead to increase the foliage yield of vegetable amaranth strains.

Path coefficient analysis was carried out using genotypic correlation coefficient among fourteen nutrients, antioxidants, yield and its contributing traits to estimate the direct and indirect effect on foliage yield. The fiber content, leaves plant^{-1} and plant height had high positive direct effect on foliage yield. High positive direct effect for fiber content, leaves plant^{-1} and plant height, moderate positive direct effect for stem base diameter Fe, Mn K and beta-carotene content in amaranth had been reported. On the other hand, high negative direct effect was observed in Ca content and negligible positive direct effect was found in Zn and protein content. Shukla *et al.* [23] also found similar results for protein content in same crop. The ascorbic acid showed negligible negative direct effect positive direct effect on foliage yield. It

was interesting that path coefficient analysis results confirmed the similarity of the correlation coefficient analysis results. Calcium had high negative direct effect and insignificant negative correlation. Potassium had considerable positive direct effect and insignificant positive correlation. Zn had negligible positive direct effect and insignificant positive correlation. Protein exhibited negligible positive direct effect and significant positive correlation. Direct selection based on these three nutrient traits (Ca, K, Zn and protein) would not be effective for the improvement of foliage yield of vegetable amaranth. Concomitant selection based on high nutrient content and high foliage yield would be effective for the improvement of vegetable amaranth. Manganese and Fe showed considerable positive direct effect with considerable positive genotypic correlation, so direct selection based on Fe and Mn would be effective for the improvement of vegetable amaranth. Beta-carotene exhibited moderate positive direct effect but its negative indirect effect via plant height made negligible genotypic correlation on foliage yield. Ascorbic acid had negligible negative direct effect with significant genotypic correlation on foliage yield. Direct selection based on antioxidant traits (beta-carotene and ascorbic acid) would not be effective for improving foliage yield. Rather, concomitant selection with high antioxidant and high foliage yield would be effective selection method for improvement of vegetable amaranth. Fiber content, leaves plant^{-1} and plant height had high positive direct effect and stem base diameter had moderate positive direct effect along with highly significant positive genotypic correlation with foliage yield. Shukla *et al.* [25] observed similar findings for plant height, fiber and beta-carotene content in vegetable amaranth. Direct selection on the basis of fiber content, leaves plant^{-1}, plant height and stem base diameter would significantly improve the foliage yield of vegetable amaranth. Selection based on plant height and leaves/plant concomitantly required considering Ca and beta-carotene content of the genotypes.

The present investigation revealed that vegetable amaranth is rich in chlorophyll *a* (290.16 µg g^{-1}), chlorophyll *b* (142.54 µg g^{-1}), Total chlorophyll (433.72 µg g^{-1}), betacyanin (302.68 ng g^{-1}) and betaxanthin (306.93 ng g^{-1}), betalain (609.53 ng g^{-1}), total carotene (89.57 mg 100 g^{-1}) ascorbic acid (83.15 mg 100 g^{-1}) and total antioxidant (21.71 TEAC µg g^{-1} dw). Five genotypes, VA14, VA16, VA18, VA15, and VA20 showed high foliage yield and also found to be a rich source of antioxidant leaf pigments and vitamins. Selection of these genotypes would be economically useful for antioxidant leaf pigments and vitamins, and high yield aspects. The genotypes VA13 and VA19 had above average foliage yield along with rich source of the antioxidant leaf pigments and vitamins while the genotypes VA2, VA3, VA9, VA11, VA12 and VA17 had a high amount of the colorant antioxidant leaf pigments and

below-average foliage yield. These eight genotypes can be used as a donor parent for integration of potential genes of the high antioxidant leaf pigments and vitamins into other genotypes.

The highest genotypic variance was observed for betalain (20318.65), followed by total chlorophyll (10522.15), betaxanthin (5157.75), betacyanin (5116.08), chlorophyll *a* (4684.08), chlorophyll *b* (2106.41) indicating greater scope of selection for these traits. Ascorbic acid (1311.99), total carotene (321.32), TAC (42.09) and foliage yield (2,52) exhibited moderate genotypic variances. The phenotypic variances for all the traits were slightly higher but close to the genotypic variances which indicated the predominance of additive gene actions. GCV values ranged from 19.41 (total chlorophyll) to 29.08% (foliage yield). The PCV values ranged from 19.70% (total chlorophyll) to 40.33% (ascorbic acid). In the present investigation, all the traits had high to moderate genotypic and phenotypic variances along with moderate GCV and PCV values, which indicate scope for improvement in these traits through selection due to predominance of additive gene action for these traits. The heritability estimates were high for all the traits and ranged from 93.76% (foliage yield) to 99.30% (chlorophyll *a*). The highest expected genetic advance was exhibited for betalain (293.64), followed by total chlorophyll (211.31%) betaxanthin (147.94), betacyanin (147.35%), chlorophyll *a* (140.99%), and chlorophyll *b* (94.44%). Genetic advance in percent of the mean (GAMP) ranged from 40.72 (chlorophyll *a*) to 80.35 (ascorbic acid). The highest GAMP was found in ascorbic acid (80.35%), followed by foliage yield (59.91%), total carotene (52.85) Chlorophyll *b* (52.16%), TAC (51.92), chlorophyll *a*, total chlorophyll, betacyanin, betaxanthin, and betalain showed moderate GAMP (around 40%). In the present study, the high heritability and high to moderate genetic advance values were observed for all the traits indicated preponderance of additive gene effects and improvement could be achieved through selection of these traits.

In the present investigation, the genotypic correlation coefficients were very much close to the corresponding phenotypic values for all the traits that indicating predominance of additive gene action i. e., less environmental influence of these traits. The chlorophyll *a* had a significant positive correlation with all the traits except total carotene and ascorbic acid. Chlorophyll *b* exhibited significant positive correlation with total chlorophyll and TAC. Similarly, total chlorophyll had a significant positive interrelationship with all the traits except total carotene and ascorbic acid. A similar trend of positive associations was observed by earlier work in *A. tricolor* [7, 143]. betacyanin had a significant positive association with betaxanthin, betalain and TAC. betaxanthin showed significant positive associations with betalain and TAC. Similarly, betalain exerted positive interrelationships with TAC. Total antioxidant capacity

showed significant positive associations with all the leaf pigments, ascorbic acid and foliage yield. These indicates that high antioxidant content was closely associated with foliage yield of vegetable amaranth. On the other hand, foliage yield had insignificant correlation with all acid. These indicate that improvement of foliage yield, ascorbic acid, antioxidant leaf pigments might be possible by improving any of the antioxidant leaf pigments. Shukla *et al.* [23] observed a positive association of foliage yield with beta carotene and ascorbic acid. Interesting results is that, ascorbic acid and total carotene had an insignificant negative and negligible interrelationship among all antioxidant vitamin and leaf pigments while it exhibited significant positive associations with total antioxidant capacity.

Antioxidant vitamins and minerals, TPC and TFC of vegetable amaranth remove free radicals from the body and help to fight against infections and other conditions including cancer, coronary artery diseases, muscular degeneration and serious eye diseases [8]. The contents of beta-carotene, vitamin C, Fe, Zn, Cu and Mn are the most important antioxidant traits of vegetable amaranth [11, 13, 15, 52, 54]. On the other hand, amaranth can relieve vitamin and nutrient deficiency in the human diet. Anemia, night blindness, scurvy, rickets and protein deficiency are serious problems for children in poor communities in third-world countries, including the Indian subcontinent. Therefore, vegetable amaranth might be an excellent source of antioxidants, nutrients and dietary fiber.

In the present study, we found that four PCs account for 98.61% of the total variation present among the 43 genotypes of amaranth, indicating that the selected antioxidant, nutrient, and agronomic traits significantly contributed to the diversity of vegetable amaranth. Shukla *et al.* [63] observed that 68% of the total variation for 16 morphological and nutritional traits was found in the first four PCs among 39 vegetable amaranth strains. PC_1 exhibited the highest positive coefficient of variation for biological yield. PC_1 also had the largest positive coefficients for foliage yield, iron, zinc, leaf area, total flavonoid content (TFC), shoot: root ratio, shoot weight, plant height, total antioxidant capacity (TAC), copper, leaves $plant^{-1}$ and manganese content whereas, this PC showed negative coefficients for beta carotene, total polyphenol content (TPC) and dietary fiber. PC_1 had a positive coefficient for all of the traits except beta-carotene, TPC, calcium and dietary fiber. PC_2, accounted for 31.72% of the variation, had the highest positive coefficient for iron and high positive coefficients for manganese, beta-carotene, TFC, and TAC. PC_2 also had the largest negative coefficient for biological yield, followed by foliage yield, leaf area, and zinc. In contrast, PC_2 had high negative coefficients for vitamin C, plant height, copper, and shoot weight. PC_3 contributed 12.82% of the genetic variation and had the highest positive coefficient of variation for zinc.

PC3 had the largest positive coefficient for manganese, iron, shoot: root ratio, copper and beta-carotene. In contrast, PC_3 had the highest negative coefficients for biological yield, foliage yield, vitamin C, TFC, leaf area, TPC, TAC, leaves plant^{-1} and plant height. Finally, PC_4 contributed only 0.90% of the total genetic variation. PC_4 had the largest positive coefficient for leaf area and high positive coefficients for vitamin C, TFC, plant height, iron, foliage yield, zinc, TPC and leaves plant^{-1}. PC_4 also had high negative coefficients for manganese, beta-carotene, biological yield, shoot: root ratio, and copper content. All of the nutrient traits and dietary fiber for PC_4 had non-significant coefficients of variation, indicating less contribution of these traits towards genetic divergence of the 43 vegetable amaranths. The results from four PCs revealed that the foliage and biological yield had a close association with all agronomic traits, indicating that a tall, thick plant having much broader leaves, heavy shoots and a high shoot: root ratio significantly increases the foliage and biological yield of the vegetable amaranth. A previous report on Amaranthus by Shukla *et al*. [63] found similar results in PC_2 and PC_3 but differed from the results we observed in PC_1 and PC4. They found that PC1 grouped the genotypes with high foliage yield but with smaller leaves plant^{-1} and PC4 grouped the genotypes with low foliage yield but broad and higher leaves plant^{-1} which may be due to the high environmental influence of related traits on foliage yield or sampling error during data collection. Although Shukla *et al.* [63] extensively investigated nutritional and morphological traits in vegetable amaranth but this is the first report of diversity study on antioxidant profile such as TPC, TFC, and TAC in combination with antioxidant vitamins, minerals, dietary fiber and agronomic traits in vegetable amaranth. Thus, the results of the antioxidant profile show that TFC has the highest contribution to TAC compared to mineral and vitamin antioxidants. Moreover, PC_1 and PC_4 distinguished those genotypes with high foliage yield, and the related agronomic traits were closely associated with high antioxidant profiles. PC_2 and PC_3, however, distinguished genotypes that had low foliage and biological yield and related traits and were also associated with a high antioxidant profile; hence, all genotypes had a high antioxidant profile. Therefore, high-yielding genotypes (especially from cluster VI) could be directly used as high antioxidant profile varieties, and low-yielding genotypes could be used as a source of donor parents in hybridization programs. All of the nutrient traits and dietary fiber results were of interest because none of the traits had a significant coefficient of variation in either the positive or negative direction, indicating less contribution of these traits to genetic divergence, but the highest contribution came from antioxidant profiles and agronomic traits.

The dendrogram of 43 vegetable amaranth genotypes for 22 antioxidant, nutrient and agronomic traits showed that the germplasm could be broadly divided into six clusters each

carrying the amaranth genotype and sharing a common gene pool. following Ward's method [157] (Fig. 1). Shukla *et al*. [63] observed six clusters in 39 vegetable amaranth genotypes, while Pandey and Singh [62] found 18 clusters in 98 grain amaranth genotypes. However, Pandey [61] divided 26 grain amaranth genotypes into 11clusters. With few exceptions, collections from the northeastern regions of Bangladesh were clearly grouped into cluster IV. Such strong relationship among diversity and geographical origin has been previously reported in amaranth [63]. Conversely, studies of oat [139] maize [140], bambara groundnut [158], and Ethiopian mustard [59] observed that genetic diversity did not follow the geographical diversity that supported the distribution of the genotypes of the rest of the clusters (clusters I, II, III, V and VI) in our study. The outcome of this analysis was consistent with the results obtained through PCA, with the major differences between the clusters attributed to the same traits that contributed the most to PC1 and PC2. Cluster VI consisted of nine genotypes of different eco-geographical regions, and among them, only three members (Accession number 16, 26 and 27) could be considered sources of genes for foliage and yield and related agronomic traits, as well as Mg and antioxidant profiles. Similarly, cluster I included 13 genotypes from different regions of Bangladesh, which were enriched with manganese, copper, calcium, and magnesium; had a moderate antioxidant profile and agronomic traits; and had a high biological yield. Moreover, cluster II consisted of a single genotype (Accession number 41) having high zinc, beta-carotene, vitamin C, TPC, calcium, protein, and dietary fiber contents; broader leaves; and high biological yield. Genotypes of these clusters might be considered sources of genes for the above-mentioned traits. In contrast, cluster III contained six genotypes from six different regions of Bangladesh and was enriched with magnesium and several antioxidants such as iron, manganese, zinc, beta-carotene, TAC, TPC, and TFC (except copper and vitamin C). Cluster IV was composed of 12 genotypes from different eco-geographical regions of Bangladesh and had high manganese, copper, beta-carotene and protein contents. Cluster V, comprising two genotypes from two different regions of Bangladesh (Accession number 20 and 21), exhibited the highest manganese, TAC, and magnesium contents and high beta-carotene, calcium and protein contents. Clusters III, IV and V might be considered donor parents for these traits. The absence of a relationship between genetic diversity and geographical diversity for clusters I, II, III, V and VI indicates that forces other than geographical origin, such as an exchange of genetic stocks, genetic drift, spontaneous variation, and natural and artificial selection, are perhaps responsible for the observed genetic diversity. Pandey [61] and Pandey and Singh [62] found similar trends in genetic and geographical diversity in grain amaranth. Our findings are in accordance with earlier reports that both PCA

and cluster analysis can disclose complex relationships between taxa in a more understandable way and with equal effectiveness [157, 159].

Conclusively, high-yielding genotypes from cluster VI could be directly used as high antioxidant profile varieties. In contrast, low-yielding genotypes having desirable genes (any clusters) for a specific trait could be used as a source of donor parents in hybridization programs. Genotypes with desirable genes of one cluster hybridized with promising genotypes of other diverge clusters could facilitate the accumulation of favorable genes in hybrids.

Amaranth was considered as the inexpensive leafy vegetables and its cultivation was also limited to Africa, South-East Asia and South America. Recently, amaranth spread over worldwide and its production and consumption have been remarkably increased due to the presence of excellent natural antioxidants such as minerals, antioxidant leaf pigments, carotenoids, vitamins, phenolics and flavonoids. These natural antioxidants have proven health benefits as they detoxify ROS in the human body and involve in defense against several diseases such as cancer, atherosclerosis, arthritis, cataracts, emphysema, retinopathy, neuro-degenerative and cardiovascular diseases [8. 48, 50]. Amaranthus species have higher mineral concentrations than commonly consumed leafy vegetables, such as spinach, lettuce and kale [178]. In *A. tricolor*, iron and zinc content is higher than that of the leaves of cassava [176] and beach pea [177]. The U.S. Department of Agriculture's National Nutrient Database for Standard Reference [243] lists a serving size of spinach as 30 g fresh weight FW (1 cup). As *Amaranthus* has higher mineral concentrations than spinach so, a serving size of leaves of 30 g FW is enough for nutritional sufficiency. In general, leafy vegetables are susceptible salt stress but amaranth is salt tolerant plant [116]. This study comprehensively evaluates the effects of varying levels of salinity stress on contents of nutrients, minerals, dietary fiber, antioxidant leaf pigments, antioxidant phytochemicals and antioxidant activities of *A. tricolor* accessions. Our results for the first time demonstrated that drought and salinity stress up to certain level significantly augment almost all these biochemical parameters in leaves of *A. tricolor*. However, the responses of these parameters to drought and salinity varied among the accessions of *A. tricolor*. Altered proteomes, enhanced vitamins and glycine betaine contents in salinity stressed *Amaranthus* have previously been reported [116, 244, 245].

One of the interesting findings of our study is that drought stresses (low, moderate and severe) significantly improved protein, ash, energy, dietary fiber, Ca, Mg, K, S, Mn, Cu, Na, Mo, B, β-carotene, ascorbic acid, total polyphenol content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC) (DPPH) and total antioxidant capacity (TAC) ($ABTS^{+}$) in leaves of *A. tricolor* compared to control condition. Similarly, salinity stresses 50 mM and 100

mM NaCl concentrations significantly improved protein, ash, energy, dietary fiber, Ca, Mg, Fe, Mn, Cu, Zn, Na, β-carotene, ascorbic acid, total polyphenol content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC) (DPPH) and total antioxidant capacity (TAC) ($ABTS^{+}$) in leaves of *A. tricolor* compared to control condition. Salt-stressed *A. tricolor* leaves also showed remarkable increment in protein, ash, energy, dietary fiber, minerals and functional antioxidant phytochemicals compared to normal cultural condition. To the best of our knowledge, this is the first report of remarkable and progressive improvement of the proximate, nutritional and functional antioxidant phytochemicals contents in *A. tricolor* under drought and salinity stresses compared to normal control cultivation and non-saline soil conditions.

The interesting finding of this study is that responses of biochemical contents in different *A. tricolor* accessions were different. The accession, VA14 under salinity stress and VA14 and VA16 under drought stress performed better in terms of most of nutrients, minerals, dietary fiber, antioxidant leaf pigments, antioxidant phytochemicals and antioxidant activities. The maturity could have a great impact on the moisture content of *A. tricolor* leaves. The moisture contents obtained in this investigation were in full agreement with the reports on sweet potato leaves by Sun *et al.* [175]. Fats are sources of omega-3 and omega-6 fatty acids. It helps in the digestion, absorption, and transport of fat-soluble vitamins A, D, E, and K. Sun *et al*. [175] observed similar results from sweet potato leaves where they mentioned that fat involved in the insulation of body organs and in the maintenance of body temperature and cell function.

As lower moisture contents of leaves are associated with higher dry matter, the salt and drought-stressed plant yielded higher dry matter compared to control or NS. The highest contents of nutrients, minerals, dietary fiber, antioxidant leaf pigments, antioxidant phytochemicals and antioxidant activities at SSS or SWS conditions and the lowest values of these plant parameters in the control or NS appears that nutrients, minerals, dietary fiber, antioxidant leaf pigments, antioxidant phytochemicals and antioxidant activities in *A. tricolor* increased by salinity and drought stress in a dose-dependent manner. The increment nutrients, minerals, dietary fiber, antioxidant leaf pigments, antioxidant phytochemicals and antioxidant activities in *A. tricolor* at MSS or MWS and SSS or SWS could be contributed to human diet in the communities of saline prone area compared to control or non-saline area. Dietary fiber has a significant role in palatability, digestibility and remedy of constipation [151]. Vegetarian and poor people in many least developed Asian and African countries used *A. tricolor* as a source of protein. Plants cultivated in SSS or SWS had progressively higher energy than those

of MSS or MWS and control or NS. However, these differences may not impact significantly on energy contribution to the human body as low amounts of this vegetable consumed in a day. Like other leafy vegetables, the low carbohydrate content of *A. tricolor* may not have a significant impact on carbohydrate contribution to the human body considering the low amount of vegetable uptake per day and a very high daily requirement for the human body.

A remarkable observation of this investigation is that the content of protein is increased with plants grown in higher doses of salinity or higher drought stress. However, the trend of fat contents in plants under salinity and drought treatment was just opposite to the contents of protein. It indicates that both salinity or drought and accession had a complex influence on carbohydrate contents in *A. tricolor* plants. In an earlier study, Petropoulos *et al*. [117] demonstrated ameliorate response in carbohydrates, protein and fat content in *Cichorium spinosum* under salinity stress. Salt stress increased the protein, dietary fiber, energy, ash and carbohydrates content and decreased moisture and fat content of *A. tricolor* accessions. Therefore, amaranth produced saline or drought prone area and coastal belt could contribute as a good source of protein and fiber in the human diet

We observed that salinity stress influences the mineral compositions of *A. tricolor* accessions. Among the tested accessions, VA14 could be consider as Ca, K, Fe, Mn, Cu and Zn enrich accession, VA3 as Mg and VA12 as Na enrich accessions. In contrast, VA16 showed better performance for all mineral elements under drought stress. In *A. tricolor*, iron and zinc content is higher than that of the leaves of cassava [247] and beach pea [248]. Similarly, Jimenez-Aguiar and Grusak [246] reported high Fe, Mn, Cu and Zn (fresh weight basis) in different *A. spp.* including *A. tricolor*. They also reported that Amaranths had higher Zn content than black nightshade, spinach and kale; more Fe and Cu content than kale. Across salinity stress, Ca, Mg, Fe, Mn, Cu, Zn and Na content were sharply and significantly increased with the increment of salinity stress and Ca, Mg, K, S, Mn, Cu, Na, Mo, B content were sharply and significantly increased with the increment of drought stress in the following order: NS or < MSS < SSS. In contrast, it was noted that the severity of salinity stress leads to a significant reduction in K content in the following order: NS or control > MSS > SSS whereas drought stress leads to a significant reduction in P, Fe, Zn content in the following order: control > LWS > MWS > SWS. Hanson *et al*. [74] found decreasing trend in Fe content in Choysum variety whereas, they found an increasing trend in Kailaan variety from wet season to dry season trial. However, Hanson *et al*. [74] found decreasing trend in Zn content both in Choysum and Kailaan variety from wet season to dry season trial. These results were fully agreed with the findings of Petropoulos *et al*. [117] that observed similar increment in Ca, Mg, Fe, Mn, Zn and

Na and decrement in K content in *C. spinosum* leaves under salinity stress. They mentioned the high content of Na should be attributed to fertilizer application and salinity treatments and suggested that the species uses Na accumulation as a means to alleviate adverse effects of salinity. With the severity of salinity stress, all the macro and micro elements except K showed increasing trend, while K showed the declining trend with the severity salinity stress. For this, amaranth cultivated in salinity prone area and coastal belt could contribute as a good source of minerals in human diet compared to normal cultivation practices.

An important finding of the current study is that beta-carotene, ascorbic acid, total polyphenol content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) of *A. tricolor* leaves were significantly augmented by the salt or drought stress at certain level. These important phytochemicals content was remarkably influenced by the accessions and accession × salt concentration interactions. The accessions VA14 could be consider as TPC, beta-carotene, TAC, ascorbic acid, antioxidant enrich accession and VA12 as flavonoid enrich accession. In case of drought stress, VA14 could be consider as TPC, beta-carotene, TFC, ascorbic acid, antioxidant enrich accession and VA16 could be consider as TAC enriched accession. In the present study, we found great variations in the tested accessions in terms of TPC, beta-carotene, TFC, TAC (DPPH) and TAC ($ABTS^+$) in different salinity or drought levels. Similarly, Alam *et al*. [122] reported pronounced variations in TFC, TPC, and TAC in different purslane accessions.

In our study, beta-carotene, ascorbic acid, TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) were significantly increased with the increment of salinity stress in the following order: NS < MSS < SSS. Similarly, we found that beta-carotene, ascorbic acid content, TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) were significantly increased with increasing soil water stress in the following order: control < LWS < MWS < SWS. These findings were partly in agreement with findings of Hanson *et al.* [74], who observed an increasing trend in beta-carotene content in Choysum variety but found a decreasing trend in beta-carotene content in Kailaan variety and decreasing trend in ascorbic acid content in both varieties from wet to dry season trial. The reason might be due to the differences in varieties. Similarly, Siracusa *et al.* [76] in buckwheat, Gharibi *et al*. [77] in *Achillea* species observed increasing trend in polyphenol, flavonoid content and antioxidant activity with the reduction of soil water content. VA14 under SSS exhibited the highest beta-carotene, ascorbic acid, TPC, TAC (DPPH) and TAC ($ABTS^+$), while VA12 under SSS had the highest TFC. In contrast, the lowest beta-carotene, TPC, TAC (DPPH) and TAC ($ABTS^+$) was observed in VA12 under NS, while VA3 under NS showed the lowest ascorbic acid and TFC. When plants fall under salinity stress, reactive oxygen

species (ROS) are produced as a result of oxidative stress. ROS induces harmful effects on plant cells. As a result, defenses against ROS are activated by generation of an array of nonenzymatic antioxidants such as ascorbic acid (AsA) and beta-carotene [97]. Salinity stress induces mevalonic acid pathway which are responsible for biosynthesis of abscisic acid (ABA) from carotenoids to counteract the osmotic stress and regulate normal plant growth and development [246]. Therefore, salinity stress enhances the accumulation of beta-carotene due to induction of ABA. AsA and αtocopherols play a crucial role in quenching intermediate/excited reactive forms of oxygen molecule directly or through catalysis of enzymes. AsA scavenges ROS (OH, SOR and $^1O_2$ directly and reduces H_2O_2 to water through ascorbate peroxidase reaction [206]. Antioxidant ascorbate and total carotenoid had vital role in counterbalancing oxidative stress and manipulating homeostasis of ROS in plants [237]. Wouyou *et al*. [245] observed ameliorate response of vitamin A and vitamin C at 90 mM NaCl concentration in *Amarantus cruentus* leaves. Similarly, Petropoulos *et al*. [117] found an elevated response to phenolics, flavonoids and antioxidant activity with the increase in salt stress in *Cichorium spinosum*. Alam *et al*. [118] observed that in purslane, different doses of salt concentrations increased total polyphenol content (TPC); total flavonoid content (TFC) and FRAP activity by 8–35%, 35% and 18–35%, respectively. Lim et al. [119] reported that buckwheat treated with 10, 50, and 100 mM after 7 d of cultivation had 57%, 121% and 153%, respectively, higher phenolic content than that of the control. Ahmed *et al*. [247] reported the increment of phenolics and TAC (FRAP) with increasing NaCl concentrations in barley. In contrast, Neffati *et al*. [248] found decrement in polyphenols and TAC (DPPH) with increasing NaCl concentrations in coriander. The increment of TPC, TFC and TAC of *A. tricolor* in response to salinity stress may be due to increase in major phenolic compounds like salisylic acid, gallic acid, vanilic acid, *p*-hydroxybenzoic acid, chlorogenic acid, *m*-coumaric acid, *trans*-cinnamic acid, iso-quercetin and rutin [212]. Previous studies have shown that biotic and abiotic stress stimulated phenylpropanoid pathway which accelerated the generation of most phenolic compounds [249, 250]. Stress-plants induce endogenous plant hormones like jasmonic acid and its methylated derivate (methyl jasmonic acid) [251]. These hormones sequentially induce phenylpropanoid pathway enzymes, including phenylalanine ammonia lyase (PAL) [252]. These enzymes accumulated the phenolic compounds.

Both at salinity and drought stress, beta-carotene showed highly significant interrelationships with ascorbic acid, TAC (DPPH), TAC ($ABTS^+$) while, this trait had significant associations with TPC and TFC. Similarly, ascorbic acid revealed significant

interrelationships with TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$). ascorbic acid interrelationships with TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$). Both beta-carotene and played a vital role in the antioxidant activity of *A. tricolor*. TPC, TFC, TAC (DPPH) significantly interrelated among each other. At drought stress, all the antioxidant pigments showed significant associations with each other. In Polyphenols and flavonoids of *A. tricolor* leaf establishing strong antioxidant activity. Alam *et al*. [118] reported the significant correlation of carotenoids, TPC, TFC with TAC (FRAP) in salt-stressed purslane. Gharibi *et al*. [77] observed positive association among TPC, TFC and antioxidant activity in drought stressed *Achillea* species.

As leafy vegetables, *A. tricolor* leaves exhibited high moisture content. Nevertheless, it demonstrated a noble source of protein, dietary fiber, carbohydrates and ash. Moisture content was significantly reduced with the increment of drought stress in the following order: (control > LDS > MDS > SDS). As lower moisture contents of leaves ensured higher dry matter, the drought-stressed plant could be a promising source of dry matter compared to control condition. In LDS, MDS and SDS, protein, ash and dietary fiber content were augmented by (17%, 17% and 4%); (80%, 29% and 21%) and (118%, 38% and 28%); respectively over control condition. However, Siracusa *et al*. [76] observed a decrease in protein content at drought stress to fully irrigated in buckwheat. The genotypic variances between two crops might be contributed for the different results. *A. tricolor* is the sources of protein for vegetarian and poor people of the third world countries. Dietary fiber has a significant role in palatability, digestibility and remedy of constipation [151]. MDS condition had the highest fat content, and the lowest fat content was observed under SDS condition. Fats are sources of omega-3 and omega-6 fatty acids. It helps in the digestion, absorption, and transport of fat-soluble vitamins A, D, E, and K. Sun *et al*. [175] observed similar results in sweet potato leaves where they mentioned that fat involved in the insulation of body organs and in the maintenance of body temperature and cell function. Control had the highest carbohydrates content and it was gradually decreased in the order: control > LDS > MDS = SDS, which was statistically similar to MDS and SDS, respectively. Carbohydrate content sharply declined with the severity of drought stress. As a leafy vegetable, the low carbohydrate content of amaranth leaves has no a substantial effect in the daily diet of the human body. As regards energy balance, SDS exhibited remarkably higher calories compared to MDS, LDS and control conditions, while these variations have no remarkable impact on the daily diet of the human body, since very little amounts were consumed in the daily diet. Drought stress increased the protein, ash, energy, fat and dietary fiber content and reduced carbohydrate and moisture content of *Amaranthus* leaves.

For this, *Amaranthus* leafy vegetable might be produced in a semi-arid and dry area in the world could be contributed as a noble source of dietary fiber and vegetarian protein in the human diet.

Amaranth leaves are noble sources of minerals (macro and microelements). The mineral content of amaranth leaves was remarkably influenced by drought stress. Zinc and Fe content of *A. tricolor* are greater than that of the cassava leaves [176] and beach pea [177]. Similarly, Jimenez-Aguiar & Grusak [178] reported high Fe, Mn, Cu and Zn (fresh weight basis) in different *A. spp.* including *A. tricolor*. They also reported that Amaranths had higher Zn content than black nightshade, spinach and kale; more Fe and Cu content than kale. Ca, Mg, K, S, Cu, Na and Mo content were sharply and remarkably augmented with the severity of drought stress from MDS and SDS conditions showing the order: control = LDS < MDS < SDS. On the other hand, Hanson *et al.* [74] reported a decline in Ca content both in Choysum and Kailaan varieties from dry season to wet season trial. SDS exhibited the highest Ca, K, S, Mg, Mn, Na, Cu, Mo and B content, while control or LDS condition had the lowest Ca, S, K, Mg, Cu, Mn, Mo, Na and B content. In contrast, control or LDS condition had the highest Zn, P, and Fe content and SDS exerted the lowest P, Zn and Fe content. Likewise, Hanson *et al*. [74] recorded a decline in Fe content of Choysum variety, whereas they reported a sharp increment in Kailaan variety from dry season to wet season trial. Moreover, Hanson *et al*. [74] recorded a remarkable increment in Zn content both in Kailaan and Choysum variety from dry season to wet season trial. Except P, Fe and Zn, all the mineral contents were progressively raised with the increment of drought stress, whereas, Zn, Fe and P were sharply declined with the increment of drought stress. Therefore, *A. tricolor* cultivated in a drought-stressed area specifically in semi-arid and drought-prone area could be contributed as a noble source of minerals content in the daily diet of the human body related to usual farming practices.

Leaf color is one of the most important parameters for consumers, playing a crucial role in choice making, preference and acceptability of the product, and may also be considered as an indicator for estimating the antioxidant properties of the leafy vegetables [259]. High redness and yellowness values recorded in the genotype VA13 could be expected since it is characterized by the presence of the high pigments (anthocyanins, carotenoids, betacyanin, betaxanthin and betalain). The results obtained in the present study were fully agreed with the results of Colonna *et al*. [259]. L*, a*, b*, chroma, betacyanin, betaxanthin, betalain, and total carotenoids were remarkably increased with the severity of salinity stress in the order, Control (No saline water) < Low salinity stress (LSS) < Moderate salinity stress (MSS) < Severe salinity stress (SSS). At LSS, MSS and SSS conditions, L*, a*, b*, chroma, betacyanin,

betaxanthin, betalain and total carotenoids were increased by (4%, 6%, 5%, 3%, 1% 2%, 0.91% & 2%), (10%, 13%, 11%, 9%, 5% 7%, 5% & 24%) and (13%, 25%, 17%, 17%, 9% 12%, 8% & 50%), respectively compared to control condition. Lim *et al*. [119] observed continuous increment in the level of carotenoids in response to all NaCl concentrations tested. They reported the greatest difference between the carotenoid content with 50 or 100 mM NaCl which was higher double than that of control sprouts, while treatment with 10 or 200 mM NaCl resulted 40% increase in carotenoids. Unlike other biotic and abiotic stresses, salinity stress induces biosynthesis of abscisic acid (ABA) from carotenoids via mevalonic acid pathway in order to regulate plant development in response to salinity tolerance. Thus, due to NaCl treatment, accumulation of carotenoids in the sprouts might be due to stimulation of the mevalonic acid pathway [119]. Alam *et al*. [118] reported both increment and decrement in total carotenoid contents in different accessions of purslane with the severity of salinity stress.

Leaf pigments of *A. tricolor* were statistically influenced by drought stress. Except total carotenoids, all the leaf pigments (Betacyanin, betaxanthin, betalain, chlorophyll *a*, chlorophyll *b* and chlorophyll *ab* content) were significantly and gradually reduced with the increasing the severity of drought stress (control > LDS > MDS > SDS). Likewise, Hsu and Kao [172] reported a decline in chlorophyll content with the increment of drought severity. They also stated that drought stress influenced growth and development of plant through osmotic stress, declining the water potential, reducing stomatal conductivity which limits CO_2 influx to leaves and unfavorable CO_2/O_2 ratio in chloroplasts, decreasing photosynthesis.

Beta-carotene, vitamin C content, TPC, TFC and TAC of *A. tricolor* were progressively influenced by drought stress or salinity stress. In this investigation, beta-carotene, vitamin C content, total polyphenol content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC) (DPPH) and TAC ($ABTS^+$) were significantly increased with the increasing of the severity of drought stress in the order: control < LDS < MDS < SDS and the severity of salinity stress were found in the order: Control < LSS < MSS < SSS. SDS or SSS condition had the highest beta-carotene, vitamin C, TPC, TFC, TAC, (DPPH) and TAC ($ABTS^+$), while the control or NS condition exhibited the lowest beta-carotene, vitamin C, TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$). Hanson *et al.* [74] reported an increase in beta-carotene content of Choysum variety. In contrast, they reported a declining trend in beta-carotene content of Kailaan variety and reduction in vitamin C content in both varieties from dry to wet season trial. The reason for reduction might be due to the genotypic variations in two different crops. Likewise, Gharibi *et al.* [77] in *Achillea* species and Siracusa *et al*. [76] in buckwheat, reported increment in antioxidant activity, polyphenol and flavonoid content with the severity of drought

stress. The ameliorate response of beta-carotene content with the severity of drought stress was also reported in Choysum varieties in dry season trial [74], and in perennial herbaceous [75]. Siracusa *et al.* [76] reported an increment of TPC, TFC in buckwheat with increasing the drought stress. Garibi *et al*. [77] also reported the enhancing response of TPC, TFC and antioxidant activity in *Achillea* species with the increment of drought stress. Similarly, Petropoulos *et al*. [117] found the elevated response of phenolics, flavonoids and antioxidant activity with the increase in salt stress in *Cichorium spinosum*. Alam *et al*. [118] reported that different levels of salinity treatment resulted 8–35% increases in TPC; about 35% increase in TFC; and 18–35% increases in FRAP activity in purslane. Lim *et al*. [119] reported that buckwheat treated with 10, 50, and 100 mM after 7 d of cultivation were 57%, 121%, and 153%, higher phenolic content than that of the control, respectively. Ahmed *et al.* [247] reported increment in phenolics and TAC (FRAP) with increasing NaCl concentrations in barley. In contrast, Neffati *et al*. [248] found decrement in polyphenols and TAC (DPPH) with increasing NaCl concentrations in coriander.

At both stresses, a total of sixteen phenolic compounds were identified including six hydroxybenzoic acids, seven hydroxycinnamic acids and three flavonoids. *Trans*-cinnamic acid was newly identified phenolic acid in *A. tricolor*. Khanam & Oba [179] in red and green amaranths and Khanam *et al*. [174] in eight different leafy vegetables including amaranths described rest fifteen phenolic acids and flavonoids with normal cultivation practices. However, an attempt was made for the first time to study the effect of drought and salinity stress in antioxidant enriched and high yield potential *A. tricolor* genotype VA3 and VA13, in terms of sixteen phenolic acids and flavonoids. Gallic acid and *p*-hydroxybenzoic acid content of the genotype VA13 under control condition and gallic acid, vanilic acid and *p*-hydroxybenzoic acid content of the genotype VA3 under control condition were higher than *A. tricolor* genotypes that reported by Khanam *et al.* [174]. Considering hydroxycinnamic acids, chlorogenic acid and *trans*-cinnamic acid were the most abundant compound followed by *m*-coumaric acid in VA13, while salicylic acid was found to be as one of the main phenolic acids followed by vanilic acid and gallic acid in VA3. A good amount of caffeic acid, *p*-coumaric acid and ferulic acid were also identified in both VA13 and VA3. The genotype VA3 had higher chlorogenic acid, caffeic acid and *m*-coumaric acid under control condition while the genotype VA13 had higher caffeic acid and *m*-coumaric acid compared to *A. tricolor* genotypes that reported by Khanam *et al*. [174]. The hydroxycinnamic acids synthesized from phenylalanine are the most extensively disseminated phenolic acids in plant tissues [180]. In plants, flavonoids occasionally occur as a glycone, although the most common forms are glycoside derivatives.

These compounds account for 60% of total dietary phenolic compounds [181]. Flavonols are the most prevalent flavonoids in the plant kingdom and glycosides of quercetin are the most predominant naturally occurring flavonols [181]. In this investigation, the flavonoids, rutin (quercetin-3-rutinoside) and isoquercetin (quercetin-3-glucoside) were the most abundant in both genotypes. The genotype VA3 and VA13 exhibited higher rutin (quercetin-3-rutinoside) content under control condition in comparison to *A. tricolor* genotypes that reported by Khanam *et al*. [174]. In both genotypes, all the phenolic acids and flavonoids had the lowest concentrations under control condition, whereas these acids exhibited the highest concentrations under SDS or SSS conditions. Hence, *A. tricolor* cultivated in a drought and salinity-stressed area specifically in the semi-arid and salt and drought-prone area could be contributed as the noble source of minerals and bioactive compounds, phenolics and flavonoid content and antioxidant activity in the daily diet of the human body related to usual farming practices.

Betacyanin, betaxanthin and betalain had highly significant positive correlations among each other and with TPC, TAC (DPPH) and TAC ($ABTS^+$). Significant association between TAC (DPPH) and TAC ($ABTS^+$) represented a crucial role of betacyanin, betaxanthin and betalain in the total antioxidant activity of *A. tricolor* leaves. Total carotenoids displayed significant relationships with beta-carotene, vitamin C, TFC, TAC (DPPH) and TAC ($ABTS^+$) demonstrating the vital role of carotenoid pigments in the antioxidant activity. beta-carotene showed highly significant interrelationships with vitamin C, TAC (DPPH) and TAC ($ABTS^+$) and significant association with TPC and TFC. It indicated that increase in beta-carotene was directly related to the increment of TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$). Similarly, vitamin C revealed significant interrelationship with TAC (DPPH) and TAC ($ABTS^+$). Both beta-carotene and vitamin C played a vital role in the antioxidant activity of *A. tricolor*. In contrast, vitamin C exerted negligible insignificant association with TPC and TFC. Jimenez-Aguilar and Grusak [178] found similar results for vitamin C in different species of *Amaranthus*. TPC, TFC and TAC (DPPH) were found significantly interrelated among each other. Alam *et al.* [118] also reported significant correlation of carotenoids, TPC, TFC with TAC (FRAP) in salt-stressed purslane. Significant positive interrelationship of TPC, TFC, TAC (DPPH) and TAC ($ABTS^+$) signify that TPC, TFC had strong antioxidant activity. Similarly, significant positive association between TAC (DPPH) and TAC ($ABTS^+$) confirmed the validation of antioxidant capacity of *A. tricolor* by two different methods of antioxidant capacity measurement. Leaf pigments, beta-carotene, vitamin C, TPC and TFC had strong antioxidant

activity as these bioactive compounds showed significant association with TAC (DPPH) and TAC ($ABTS^+$).

In the present study, several adaptive responses were observed in *Amaranthus tricolor* cultivars under four water deficit conditions. Tested cultivars exhibited some morphological, physiological and biochemical changes under different levels of drought. The tested cultivars had tremendous variability among studied ROS markers, compatible solutes and non-enzymatic antioxidant parameters. Among studied cultivars, VA14 and VA16 had higher biomass, SLA, chlorophyll content, RWC, compatible solutes and non-enzymatic antioxidant like, total carotenoids, free ascorbic acid, proline, TPC, TFC and TAC and lower oxidative stress responses due to less accumulation of stress markers like, MDA, H_2O_2 and EL related to higher water use efficiency could be identified as a drought tolerant cultivar. These two cultivars could be used as drought tolerant cultivars or selected as tolerant parents to obtain more tolerant cultivar in hybridization programs.

Growth (biomass production) are the primary processes to be affected by drought [85]. In our investigation, growth reduction was observed under moderate and severe stress. This suggests that even at reduced soil water availability, *Amaranthus tricolor* cultivars are able to grow. Our results agree with the findings of Achten *et al.* [188] in *J. curcas* who have observed that water withhold would arrest growth but maintaining plants at low soil water availability (40%) would allow them to continue growing, although at a slower rate than fully irrigated condition. Total biomass of all *A. tricolor* cultivars significantly reduced when exposed to drought stress, in terms of drought severity dependent manner, which indicated that drought stress depressed plant growth. Reduced biomass under severity of drought treatment could be attributed by inhibition of cell elongation and expansion, reduced turgor pressure, alteration of energy from growth to synthesis of compatible solutes to maintain cell turgor reduced water uptake resulting in a decrease in tissue water contents and trimming down the photo-assimilation and metabolites required for cell division [189]. VA14 and VA16 had less growth inhibition and the highest sustainability in production compared to other cultivars under drought stress which suggested tolerance to drought. Our study demonstrated that SLA, an indicator of leaf thickness, had a significant decrease to the severity of drought stress in all cultivars. The reduction was observed under moderate and severe stress. This suggests that even at reduced soil water availability, *Amaranthus tricolor* cultivars are able to maintain SLA. Similar trend of decline in SLA was observed by Guerfel *et al.* [190]. VA14 and VA16 exhibited higher SLA compared with other cultivars, suggesting the better performance to accumulate more dry mass per unit of leaf area under drought stress. We observed the positive

relationship between SLA and total biomass (r = 0.86** Supplemental Table S1), suggested that salinity may also influence plant growth through reduction in specific leaf area.

Relative water content is useful variables to evaluate the physiological water status of plants and its metabolic activity and survival that could be used as an attribute for discriminating tolerant and sensitive plants under water deficit [191]. *Amaranthus tricolor* cultivars established drought-induced reduction of RWC with the severity of drought ($P < 0.01$). RWC reduction was observed under moderate and severe stress. This suggests that even at reduced soil water availability, *Amaranthus tricolor* cultivars are able to maintain RWC. Reduced turgor pressure, reduced water uptake due to shortage of available water in the soil caused by drought, hinder water uptake by roots, resulting in decrease of RWC in the leaves. In this investigation, a highly negative correlation between RWC and both proline and soluble protein contents was observed (r = -0.63**, r = -0.55* Supplemental Table S1). It has also been extensively documented in several species that compatible solutes like free proline and soluble protein accumulation facilitate osmoregulation under drought stress [192]. VA14 and VA16 exhibited the higher leaf RWC under drought stress conditions, could be elucidated by a potential osmoregulation strategy due to the higher accumulation of compatible solutes in comparison to another cultivar studied. Thus, these two cultivars seem to be more efficient in terms of decreasing the cellular osmotic potential allowing the roots to absorb a sufficient amount of water to maintain cell turgidity and for improving potentiality in hydration status.

There was a significant difference in photosynthetic leaf pigment (chlorophyll *a*, *b* & *ab*) ($p < 0.01$) among all soil water deficit treatments and among all amaranth varieties. An increase in water deficit stress inhibiting chlorophyll synthesis which is supposed to occur at four consecutive stages: (I) the formation of 5-aminole-vuliniuc acid (ALA); (II) ALA condensation into porphobilinogen and primary tetrapyrrol, which is transformed into protochlorophyllide; (III) light-dependent conversion of protochlorophyllide into chlorophyllide; and (IV) synthesis of chlorophylls *a* and *b* along with their inclusion into developing pigment–protein complexes of the photosynthetic apparatus [193]. The observed decrease in photosynthetic leaf pigment under drought stress, also associated to free radical-induced oxidation of chlorophyll pigment [194], disruption of some chloroplasts or a consequence of increased activity of chlorophyll degrading enzyme, chlorophyllase [195]. Lutts *et al*. [196] indicated that chlorophyll concentration in stressed tissues can be construed as an index of tissue tolerance to drought. In this study, VA16 and VA14 having more chlorophyll content than the other studied cultivars, it could be suggested that these cultivars are more drought tolerant than the others. The reduction of photosynthetic leaf pigment was

found lower in all cultivars, this may be due to present of antioxidant leaf pigment betalain (Betacyanin and betaxanthin) that absorbed significant amount of radiation and protected the drought stressed chloroplasts from harmful excessive light. These results were fully agreement with the results of Jain *et al.* [197]. They found that high betalain content in *Disphyma australe* showed physiologically more tolerant to salt stress. Moreover, betalain protects the drought stressed chloroplasts by reducing the ROS in thailakoids [198].

Drought stresses aggravate the production of ROS like superoxide, hydrogen peroxide, hydroxyl radicals, alkoxy radicals, singlet oxygen etc. resulting in oxidative damage in cell [199]. Mechanisms of ROS generation in biological systems are electron reduction (O_2) at higher oxygen concentrations, initial activation of O_2 by xanthine oxidase, dis-mutation of the superoxide anion by superoxide dismutase to yield H_2O_2. ROS may react with proteins, lipids and DNA, causing oxidative damage and impairing the normal functions of cells. Various organelles including chloroplasts, mitochondria and peroxisomes are the seats as well as first target of ROS produced under drought stress [189]. In MDS and SDS, both MDA and H_2O_2 content was remarkably increased that agreed with the results those observed in strawberry [200]. In the present investigation, extreme accumulation of H_2O_2 in MDS and SDS might have accelerated the Haber-Weiss reaction, resulting in hydroxyl radical (OH•) formation and therefore, resulting in serious lipid peroxidation and cell membrane damage [103]. The static ROS content from control to LDS might be due to inhibition of ROS generation in plant tissues and up-regulates ROS scavenging activity by active accumulation of excessive proline, total carotenoid, ascorbic acid, TPC, TFC and antioxidant activity that inhibited the increment of MDA and H_2O_2 content. Although the highest accumulation of proline, total carotenoid, ascorbic acid, TPC, TFC, antioxidant activity was noted in SDS condition compared to any stresses, but MDA and H_2O_2 accumulation was also the highest. This might be likely that the vegetable amaranth fell in to severe stress and could not cope with damage caused by drought. Maintaining a balance between ROS production and scavenging is crucial under stressed conditions [201]. In our study, compatible solutes and non-enzymatic antioxidants like proline, total carotenoid, ascorbic acid, TPC, TFC and antioxidant activity was significantly increased as a protective mechanism under drought-stressed conditions from LDS to SDS to reduce the H_2O_2 and MDA accumulation. The exposure of four cultivars in MDS and SDS exhibited differential increment of H_2O_2 and MDA accumulation, this might be due to the differential responses of ROS (H_2O_2, MDA) scavenging ability of these cultivars. Under MDS and SDS, less tolerant cultivar VA6 showed the highest H_2O_2 and MDA content showing that this cultivar experienced more lipid peroxidation and higher levels of cellular damage (Fig. 2a, 2b). This

cultivar also had the lowest compatible solutes and non-enzymatic antioxidant like proline total carotenoid, ascorbic acid, TPC TFC and antioxidant activity compared to any cultivars. In contrast, tolerant cultivars VA16 and VA14 had low H_2O_2 and MDA content and alleviated the oxidative stress through transcriptional regulation of multiple defense pathways, such as compatible solutes and non-enzymatic antioxidant, antioxidant enzymes and the ASC-GSH cycle and improved the effects caused by drought stress through protecting ROS biosynthesis. (See compatible solute accumulation and non-enzymatic antioxidant section). Enhanced electrolyte leakage is considered to be a sign of destruction and deterioration under water stress. In this study, electrolyte leakage was remarkably increased with the drought severity. It indicated that cell electrolyte leakage could be used as a criterion to differentiate stress tolerant and susceptible cultivars and that in some cases lower electrolyte leakage could be correlated with abiotic stress tolerance. Furthermore, the observed dramatic increase in MDA and H_2O_2 under drought severity induced cellular membrane damage, which is demonstrated by an increase in EL. VA14 and VA16 showed lower electrolyte leakage can be used as tolerant cultivars. Moreover, MDA H_2O_2 and EL showed the strong negative correlation observed with total biomass (r = -0.91**, r = -0.89** and -0.79** Supplemental Table S1) suggests that the drought induced lipid peroxidation and H_2O_2 generation oxidative stress can be one of the reasons for inhibition of biomass production in *A. tricolor* plants.

One of the most common stress tolerance strategies in plants is the overproduction of different types of compatible organic solutes. Generally, they protect plants from stress through different means such as contribution towards osmotic adjustment, detoxification of ROS, stabilization of membranes, and native structures of enzymes and proteins [189]. In this study, the proline content of the leaves had significant ($p < 0.01$) and remarkable increase across all cultivars under all drought stresses. Highly significant negative correlations between the proline and soluble protein content in *Amaranthus tricolor* leaves and the biomass production (r = -0.66** and -0.61**, respectively), were observed (Supplemental Table S1). The highest proline accumulation in response to drought stress observed in VA14 and VA16 might be related to their competitive ability in a drought against oxidative stress. Proline have antioxidant activity, activates detoxification systems, contributes to cellular homeostasis by protecting the redox balance, and functions as protein precursor, an energy source for the stress recovery process (See ROS markers section). It mainly involved in protection against oxidative stress thus reduced lipid peroxidation resulting in in different plant species and had an essential role in stabilizing proteins and cellular membranes in plant cells in presence of high levels of osmolytes. In addition, proline induces expression of stress-induced responsive genes, activates

antioxidant enzymes [202]. Proline protects photosynthetic apparatus. In our study, proline accumulation is high that resulted in less decline of chlorophyll content (see photosynthetic leaf pigment section). Synthesis of stress proteins is a ubiquitous response to cope with prevailing stressful conditions including water deficit. Most of the stress proteins are soluble in water and therefore contribute towards the stress tolerance phenomena by hydration of cellular structures [189]. In our study, soluble protein didn't have any role in *A. tricolor* cultivar except of susceptible cultivar VA6 that had increasing trend of soluble protein.

Carotenoids have received little attention despite their capacity to scavenge singlet oxygen and lipid peroxy-radicals, as well as to inhibit lipid peroxidation and superoxide generation under dehydrative forces. A major protective role of carotenoids and beta-carotene in photosynthetic tissue may be through direct quenching of triplet chlorophyll, which prevents the generation of singlet oxygen and protects from oxidative damage and help plants to withstand adversaries of drought [189]. Total carotenoid is a lipophilic antioxidant and are able to detoxify various forms ROS [203]. Plants are able to release of excessive energy by thermal dissipation associated with an increase in the total carotenoid concentration in water stressed plants. This can be attributed to the activation of the xanthophyll cycle. Thus, presumed that the role of antioxidants and beta-carotene pigment in regulating photosynthetic electron transport is crucial [204]. Ascorbic acid (AA) is one of the powerful antioxidants [205]. Ascorbic acid along with vitamin E plays a key role in quenching intermediate/excited reactive forms of molecular oxygen either directly or through enzymatic catalysis. It allows enzymatic and non-enzymatic antioxidant defense system and thereby increased efficiency and contribution to ROS neutralization and balance. AA can directly scavenge superoxide, hydroxyl radicals and singlet oxygen and diminish H_2O_2 to water via ascorbate peroxidase reaction [206]. Recently, dehydroascorbic acid has emerged as a signaling molecule regulating stomatal closure [207]. In this study, both total carotenoid and ascorbic acid had significant and remarkable ($p < 0.01$) increment across drought stresses and cultivars. These results were fully agreed with the results of Choysum in dry season trial by Hanson *et al*. [74], where, they found the elevated response of total carotenoid and ascorbic acid, respectively from control to drought stress. The highest total carotenoid and ascorbic acid was observed in VA16 and VA14, respectively under all water treatment conditions while, VA6 and VA11 exhibited the lowest total carotenoid and ascorbic acid, respectively (Fig. 4a, 4b). The increased content of ascorbic acid, indicates the crucial role of the ASC–GSH cycle for scavenging ROS in leaves of *A. tricolor*. Similarly, the drought tolerant, but not the sensitive cultivar, accumulated higher activities and transcripts of the ASC–GSH cycle.

Generally, accumulation of polyphenols which possess antioxidant properties is stimulated in response of ROS increases under biotic and abiotic stresses. They are plentiful present in plant tissues [205]. Polyphenols can chelate transition metal ions, can directly scavenge molecular species of active oxygen, and may quench lipid peroxidation by trapping the lipid alkoxyl radical. Furthermore, flavonoids and phenylpropanoids are oxidized by peroxidase, and act in H_2O_2-scavenging, phenolic/AsA/POD system. In the present study, the increment of total polyphenol (TPC) and flavonoid content (TFC) were depended on degree of water stress (Fig 4c, 4d)). Reddy *et al.* [199] in higher plant reported ameliorate response of TPC and TFC under drought stress.

Total antioxidant activity is the combined results of all enzymatic and non-enzymatic antioxidants activity in natural and/or biotic/abiotic stress. Tolerant plant genotypes usually have a better antioxidant content to protect them from oxidative stress by maintaining high antioxidant enzyme and antioxidant molecule activity and contents under stress conditions. Antioxidants protect the cells from free radicals and therefore have been considered as a method to improve plant defense responses [208]. Water stress can lead to elevation of reactive oxygen species and, therefore, higher amounts of antioxidants is required to compensate stress condition and increase the tolerance [209]. Antioxidant activity has a crucial role in maintaining the balance between the production and scavenging of free radicals [210]. The observed positive correlations among total carotenoid, ascorbic acid, TPC, TFC and TAC (see supplementary Table S1) indicated that the increase in any one of these antioxidant activities was accompanied by an enhancement in each of the five antioxidant activity, presumably as a result of high demand for quenching H_2O_2. It can most likely be inferred that total carotenoid, ascorbic acid, TPC, TFC and TAC correspondingly organize in relation to each other.

The results of the present investigation suggested that *A. tricolor* is tolerant to drought stress. We select one tolerant and one sensitive *A. tricolor* genotype previously screened for drought stress based on morphological and physiological traits to elucidate key non-enzymatic, physiological, and antioxidant enzymatic defense mechanisms involved. The above defense mechanisms significantly varied in the tolerant and sensitive varieties as are discussed in detail in the following sections.

Growth is a primary process that affects drought [85]. Total biomass of both varieties of *A. tricolor* significantly declined to MDS and SDS conditions, in comparison with control treatment, indicating that drought stress declined the growth of both varieties. Whereas the tolerant variety showed less decline in total biomass. These results were in full agreement with the results of Sekmen *et al*. [220] who observed that the growth rate of tolerant M-503 cultivar

was less affected from drought treatments as compared to the sensitive 84-S cultivar. In our earlier study, we observed decrease in RWC and biomass reduction with the increment of drought stress [213]. Previous studies also have shown that drought stress inhibited growth and RWC in strawberry [97], xerophyte *Capparis* ovata [221] and cotton [220]. It might be accredited to prevent cell elongation and expansion [222, 223], reduction of turgor pressure, changes of energy from growth to biosynthesis of metabolites to preserve turgor pressure of cell, declines in absorption of water that ultimately reduces water content of cell and nitrogen assimilation [224, 225], reduces the photo-assimilation [226] and metabolites for cell division [189]. In the present investigation, an indicator of leaf thickness SLA had a sharp decline with the increment of drought stress in both varieties under MDS and SDS conditions in comparison with control treatment. Guerfel *et al.* in olive [190] and Sarker and Oba in *Amaranthus* [213] observed a similar trend of decline in SLA. Growth and SLA reduction in sensitive genotype VA15 were significantly higher than that of tolerant genotype VA13 under both MDS and SDS conditions i.e., VA13 showed better adaptation compared to VA15. Similarly, Zheng *et al*. [227] also found different adaptation in two genotypes of *C. bungee.* In this study, VA13 had more chlorophylls content and less decline in chlorophylls than VA15, suggesting that VA13 was more drought tolerant compared to VA15. Sarker and Oba [213] in *A. tricolor*, Shahbaz *et al*. [228] in wheat and Zhang and Kirkham [214] in sorghum and sunflower observed a decline in leaf chlorophyll contents under drought stress conditions. Drought stress induces the oxidation of chlorophyll pigment resulting in decrement of chlorophyll pigments [194], chloroplasts disruption or augmented activity of chlorophyllase [195]. *A. tricolor* has betacyanin and betaxanthin that absorb a substantial amount of radiation which ultimately protects chloroplasts from harmful excessive light under stressful condition [198]. In the present study, this might be the reason for lower chlorophyll reduction in both varieties. RWC is convenient attributes for assessing physiological hydration condition of crops and its metabolism and existence. It might be utilized for distinguishing between sensitivity and tolerance in drought-stressed crops [191]. Both *A. tricolor* varieties resulted in a drought-induced reduction of RWC under MDS and SDS conditions, compared to the control treatment, respectively, however, the reduction was more drastic in VA15 compared to VA13. In our previous studies, we also found similar results in *A.* tricolor [213]. Munne-Bosch and Penuelas [97] in strawberry, Ozkur *et al*. [221] in xerophyte *Capparis ovata* and Sekmen *et al*. [220] in cotton observed a similar decline in RWC under drought stress. Drought stress reduces turgor pressure, decreases available water in the soil, hampers roots water absorption, finally results in decrease in RWC of leaves. Under drought stress, VA13 exhibited

higher RWC; it might be due to probable osmoregulation approach and greater antioxidants accumulation under drought stress in comparison to VA15 [213]. Turkan *et al*. [229] and Cia *et al*. [230] showed that tolerant varieties have maintained better RWC under drought stress. Thus, VA13 seemed to be more capable to decrease the cellular osmotic pressure and to permit the roots for absorbing adequate water to sustain cell turgor pressure and for taming potentiality against hydration status.

Drought stresses intensify the manufacture of ROS like alkoxy radicals, $O_2^{\bullet-}$, singlet oxygen, H_2O_2, OH• etc. which ultimately create oxidative stress in cell [213, 231]. Primary stimulation of O_2 by xanthine oxidase, $O_2^{\bullet-}$ dismutation, electron reduction at higher O_2 level are the main mechanisms of ROS generation in plants [232]. ROS causes oxidative stress through damage DNA, lipids and proteins, Restricting the normal cell functions. Drought stress aggravates ROS production in chloroplasts, mitochondria and peroxisomes [86,189]. In our study, we found a substantial production of H_2O_2, lipid peroxidation and increase in EL in the sensitive variety (VA15) of *A. tricolor* under drought stress. EL leakage was much greater in the sensitive variety (VA15) as compared to the tolerant variety (VA13). These results agreed with the results of our previous study in amaranth [213], Christou *et al*. [200] in strawberry and Chakraborty *et al*. [232] in groundnut. Our results clearly demonstrated that at similar drought stress, the sensitive *A. tricolor* accumulated more ROS compared to the tolerant variety. Hence the tolerant variety maintained the ROS to a relatively lower level than sensitive variety. In the present investigation, extreme accumulation of H_2O_2 at MDS and SDS in the sensitive variety might be due to acceleration of the Haber-Weiss reaction that causing formation of hydroxyl radical (•OH), hence, resulting in more MDA production and damage of cell membrane [89]. At stressful conditions, it is crucial to maintaining a balance between ROS assembly and detoxification [201]. In our study, drought-stressed conditions remarkably augmented non-enzymatic and enzymatic antioxidants by defensive techniques from MDS to SDS to lessen EL, H_2O_2 and MDA accumulation. The tolerant cultivars VA13 had very low H_2O_2 and MDA content. The tolerant cultivar improved the stressful condition by several protection ways, such as non-enzymatic antioxidant, antioxidant enzymes and AsA-GSH cycle which inhibited drought stress impact by protection of ROS generation. Under water stress, electrolyte leakage is considered to be a symbol of damage and descent [233]. In the present investigation, drought stress progressively enhanced electrolyte leakage. Hence, electrolyte leakage might be used to distinguish stress-susceptible and tolerant cultivars. Abiotic stress tolerance is associated with lower electrolyte leakage. The Severity of drought-induced progressive increment in MDA and H_2O_2 that enhanced the damage of cell membrane in the sensitive variety and demonstrated by

a sharp increase in EL. Tolerant genotype VA13 showed lower electrolyte leakage compared to sensitive genotype.

Proline content of both the varieties was significantly increased under MDS and SDS conditions, whereas the increment was greater in the sensitive variety VA15 compared to tolerant variety VA13. It is evident from the results that proline had no significant role in the mechanisms of drought stress tolerance in *A. tricolor*, as a functional osmolyte and antioxidant for adjustment of osmotic stress and ROS detoxification in *A. tricolor* as it accumulates to higher levels in the drought-sensitive variety. Nayyar and Walia [234] and Tatar and Gevrek [235] in wheat, Zheng *et al*. [227] *Catalpa bungee* observed proline increment under drought stress. Carotenoids are capable to scavenge lipid peroxy-radicals and singlet oxygen and inhibit superoxide generation and lipid peroxidation under drought stress [189]. Total carotenoids are lipophilic antioxidants that are capable to purify different types of ROS [203]. In plants, total carotenoid usually absorbs light at 400 and 550 nm and transfer the apprehended energy to the chlorophyll [236]. Carotenoids can act as an antioxidant that inhibits oxidative damage by scavenging $^1O_2$, quenching triplet sensitizer (3Chl*), exciting chlorophyll (Chl*) and protecting the photosynthetic apparatus. Ascorbate (AsA) is one of the powerful antioxidants [205]. AsA and αtocopherols predominately quench O_2 straightly or by enzymes catalysis. It permits non-enzymatic and enzymatic antioxidative ROS detoxification. AsA scavenges OH, SOR and $^1O_2$ directly and reduces H_2O_2 to water through ascorbate peroxidase reaction [206]. Antioxidant ascorbate and total carotenoid had a vital role in counterbalancing oxidative stress and manipulating homeostasis of ROS in plants [237]. Our results showed that the total carotenoid level was increased in VA13, while the decrement of this compound was observed in VA15. In the tolerant variety VA13, had a remarkable rise in ascorbate-glutathione content and ascorbate-glutathione redox status, while the sensitive variety VA15 exhibited negligible increment of ascorbate-glutathione content and ascorbate-glutathione redox status. For instance, drought and salt stress increased the activity of ascorbate-glutathione content and ascorbate-glutathione redox status in pea [238], wheat [239], sorghum and sunflower [214], *Catalpa bungee* [227], strawberry [97] and groundnut [232], particularly for tolerant lines under water deprivation condition. The AsA-GSH content, AsA-GSH redox status specifies the essential part of the AsA–GSH cycle for detoxification of ROS in the tolerant *A. tricolor.* Similarly, Hernandez *et al.* [238] reported that salinity stress accumulated higher transcripts of the AsA–GSH cycle in the tolerant variety compared to the sensitive variety.

Drought stress generated superoxide from photosynthetic and respiratory electron leakage in chloroplast. Superoxide dismutase (SOD) enzyme dismutated superoxide into H_2O_2.

H_2O_2 was decomposed by different peroxidases such as ascorbate peroxidase (APX), glutathione peroxidase (GPX) and phenol peroxidase [86] into the water by using various reducing agents. In contrast, catalase (CAT) mostly decomposed photorespiration mediated H_2O_2 in the peroxisome [91]. In this study, we found that drought stress induced CAT and SOD activities in both varieties whereas, CAT and SOD activities were much greater in the tolerant variety VA13 compared to sensitive variety VA15, suggesting role of CAT and SOD in drought tolerance in *A tricolor* by detoxification of H_2O_2 and activating dismutation reaction to alter SOR to hydrogen peroxide, respectively. These results agreed to results of Ben Amor et al. in halophyte *Cakile maritima* [240] where they interrelated in increased SOD activity with plant salt tolerance. Khanna-Chopra and Selote [239] in wheat, Ozkur *et al*. [221] in *Capparis ovata*, Zhang and Kirkham [214] in sorghum and sunflower and Chakraborty *et al*. [232] in groundnut observed enhanced activities of SOD, POX and CAT under drought and salt stress. Sekmen *et al*. [220] found that the sensitive genotype 84-S associated with decreased activities of catalase (CAT) and peroxidase (POX) to combined stress while the tolerant genotype M-503 was associated with higher activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX) and induced CAT and POX at combined drought and heat stress. In contrast, GPOX had significant and remarkable increasing activity under drought stress, in both varieties, while sensitive variety, VA15 exhibited the highest increase compared to VA13 at all drought stress treatments. Drought stress accelerated higher GPOX increase in the sensitive variety compared to the tolerant variety; it is clearly evident that GPOX had a significant role in enhancing APX activity in the sensitive variety at greater H_2O_2 concentration. There was a slight and negligible increase in GR, MDHAR, APX and DHAR activity in sensitive variety VA15 under drought stress, while tolerant variety VA13 exhibited the greatest dramatic increase in GR, MDHAR, APX and DHAR activity under drought stress. Hernandez *et al.* [238] in pea found increased activities of GR, MDHAR, APX and DHAR while Chakraborty *et al*. [232] in groundnut showed APX increment under salt stress. Similarly, Khanna-Chopra and Selote [239] and Ozkur *et al*. [221] found that increased activities of APX and GR were associated with drought stress. It indicated that at lower H_2O_2 load, GR, MDHAR, APX and DHAR performed as a main ROS scavenging enzyme in *A. tricolor* under drought stress that may have related to satisfactory regulation of H_2O_2 in the tolerant variety VA13. Increase in AsA-GSH content, reduced AsA-GSH redox status accompanied by AsA–GSH cycle enzymes such as GR, MDHAR, APX and DHAR, clearly evident that AsA–GSH cycle played a crucial role for scavenging ROS in the tolerant variety of *A. tricolor.* Abogadallah *et al*. [241] reported APX-

GR as the main H_2O_2 detoxifier at low H_2O_2 load and performed as a satisfactory controller for ROS balancing in barnyard grass under salt stress.

SUMMARY

47 vegetable amaranth genotypes from different eco-geographic regions of Bangladesh were evaluated under two separate sub-experiments to investigate variability and diversity in nutritional such as antioxidant vitamins and minerals composition, protein, leaf quality such as fiber, yield and yield contributing morphological traits. Genetic variability and diversity were studied in a Randomized Complete Block Design (RCBD) with three replications at Bangabandhu Sheikh Mujibur Rahman Agricultural University in Bangladesh. The experiments were conducted to study the degree of genetic parameters, associations among different traits, and the direct and indirect contribution of different traits towards foliage yield. The analysis of variances for all the traits were found highly significant indicating wide range of genetic variability and diversity among traits. High mean value, high range of variability and high genotypic variance were observed for all the traits except content of Ca, protein and beta-carotene. Vegetable amaranth was rich in iron, zinc, manganese, magnesium and potassium. Ten strains gave the best (more than 5 kg) foliage yield with rich in antioxidant minerals and vitamins. Selection of these genotypes would be economically useful for antioxidant vitamins, minerals and yield aspects. On the other hand, eight genotypes had high amounts of antioxidant vitamins and minerals with below average foliage yield and could be utilized as donor parents for introgression of genes in vitamins and minerals deficient lines. Close differences between genotypic and phenotypic variances and genotypic and phenotypic coefficients of variation were observed for all the traits.

High to moderate genotypic coefficients of variation and heritability coupled with high to moderate genetic advance in percent of mean was observed for all the traits. Considering genetic parameters K, Fe, Zn, Mn, ascorbic acid, plant height, diameter of stem base, leaves plant^{-1}, fiber content and foliage yield would be selected for the improvement of vegetable amaranth genotypes under study. However, correlation study revealed that selection based on Fe, Mn, ascorbic acid, protein, fiber content, plant height, leaves plant^{-1} and stem base diameter could lead to increase in foliage yield of vegetable amaranth genotypes. Insignificant genotypic correlations between foliage yield with most of the antioxidant vitamins and minerals traits indicating that selection for high vitamins and minerals content might be possible without compromising yield loss. Therefore, concomitant selection for high nutrient, antioxidant and high foliage yield would be effective for improvement of the vegetable amaranth. Based on mean, genetic parameters and correlation coefficient values, five vegetable amaranth genotypes i. e., AA19, AA10, AA3, AA24 and AA7 might be selected as high vitamin

and minerals containing high yielding vegetable amaranth varieties. Fiber content, leaves plant^{-1}, plant height, and stem base diameter had high positive direct effects and Fe, Mn, and carotenoid exhibited moderate positive direct effects on foliage yield. Based on mean, range, genetic parameters, correlation coefficient and path coefficient values, direct selection through Fe, Mn, fiber content, plant height and diameter of stem base, leaves plant^{-1} would significantly improve the foliage yield of vegetable amaranth. On the other hand, concomitant selection based on high nutrient and antioxidant content and high foliage yield would be effective selection method for improvement of vegetable amaranth.

Forty-three vegetable amaranth (*Amaranthus tricolor* L.) genotypes were selected from 102 genotypes based on our previous studies. The genotypes were evaluated under four separate sub-experiments for genetic variability, diversity, heritability, genetic association among mineral elements, quality and agronomic traits as well as for genetic variability in terms of total antioxidant capacity, antioxidant leaf pigments vitamins and foliage yield in randomized complete block design (RCBD). Vegetable amaranth was a rich source of K, Ca, Mg, proteins and dietary fiber with average values among the 43 genotypes (1.014%, 2.476%, 2.984, 1.258% and 7.81%, respectively). It was also rich in chlorophylls, betacyanin, betaxanthin, betalain, carotene, ascorbic acid and total antioxidant. Six genotypes (VA13, VA14, VA16, VA18, VA26, VA27) showed a biological yield > 2000 g/m^2 and high mineral, protein and dietary fiber contents; eleven genotypes had high amount of minerals, protein and dietary fiber with above average biological yield; nine genotypes had below average biological yield but were rich in minerals, protein and dietary fiber. The genotypes VA14, VA16, VA18, VA15, and VA20 could be selected as amaranth vegetable varieties with high yields and abundance antioxidant leaf pigments and vitamins to produce juice. The genotypes VA13 and VA19 had above-average foliage yield and high antioxidant profiles while the genotypes VA2, VA3, VA9, VA11, VA12, and VA17 had a high antioxidant profiles and below-average foliage yield. These genotypes could be used as a donor parent for integration of potential high antioxidant profiles genes into other genotypes. The correlation study revealed a strong positive association among leaf area, shoot weight, shoot/root weight, stem base diameter, all the antioxidant leaf pigments, total antioxidant capacity and foliage yield. Insignificant genotypic correlation was observed among mineral, quality and agronomic traits, except K vs. Mg, protein vs. dietary fiber and stem base diameter vs. Ca. Total carotene and ascorbic acid exhibited insignificant genotypic correlation with all the traits except total antioxidant capacity. This indicates that selection for mineral, protein, dietary fiber content, antioxidant vitamins might be possible without compromising yield loss. On the other hand, most of the

interrelationships among antioxidant leaf pigments traits indicated that improving of one antioxidant leaf pigment significantly improved the other antioxidant leaf pigments.

On the other hand, these genotypes were evaluated to determine the status of total antioxidant content, polyphenol, flavonoid, antioxidant vitamins and minerals, dietary fiber, nutritional and agronomic traits and the magnitude of genetic diversity based on the contribution of those traits for meaningful grouping and proper utilization in future breeding program. Multivariate (Principal component and cluster) analysis was done using numerical taxonomic techniques of Sneath, & Sokal. Four principal components contributed 98.61% of the variation. Biological yield and total antioxidant content was strongly associated with their related all agronomic traits. Total flavonoid content had a higher contribution to total antioxidant capacity compared to vitamin and mineral antioxidants. Contribution of antioxidant profile and agronomic traits was the highest in diversity of vegetable amaranth. Both high and low yielding genotypes had a high antioxidant profile. Therefore, high yielding genotypes (From cluster VI) could be used directly as high antioxidant profile varieties and low yielding genotypes as a source of donor parents in hybridization program. Cluster analysis grouped the genotypes into six clusters. The diverse genotypes in different clusters were identified. Genotypes with desirable genes of one cluster hybridized with promising genotypes of other diverge clusters could facilitate the accumulation of favorable genes in hybrids.

Four selected vegetable amaranths were grown under four soil water content to evaluate their response in nutrients, minerals, antioxidant leaf pigments, vitamins, polyphenol, flavonoid and total antioxidant activity (TAC). Vegetable amaranth was significantly affected by variety, soil water content and variety × soil water content interactions for all the traits studied. Increase in water stress, resulted in significant changes in proximate compositions, minerals (macro and micro), leaf pigments, vitamin, total polyphenol content (TPC), and total flavonoid content (TFC) of vegetable amaranth. Accessions VA14 and VA16 performed better for all the traits studied. Correlation study revealed a strong antioxidant scavenging activity of leaf pigments, ascorbic acid, TPC and TFC. Vegetable amaranth can tolerate soil water stress without compromising the high quality of the final product in terms of nutrients and antioxidant profiles. Therefore, it could be a promising alternative crop in semi-arid and dry areas and also during dry seasons.

Bioactive compounds, vitamins, phenolic acids, flavonoids of *A. tricolor* are the sources of natural antioxidant that had a great importance for the food industry as these detoxify ROS in the human body. These natural antioxidants protect human from many diseases such as cancer, arthritis, emphysema, retinopathy, neuro-degenerative cardiovascular diseases,

atherosclerosis and cataracts. Moreover, previous literature has shown that drought stress elevated bioactive compounds, vitamins, phenolics, flavonoids and antioxidant activity in many leafy vegetables. Hence, previous literature grew much interest to study nutritional and bioactive compounds, phenolic acids, flavonoids and antioxidant capacity of amaranth under drought stress for evaluation of the significant contribution of these compounds in the human diet. The genotype VA3 was assessed at four drought stress levels that significantly affected nutritional and bioactive compounds, phenolic acids, flavonoids and antioxidant capacity. Protein, ash, energy, dietary fiber, Ca, K, Cu, S, Mg, Mn, Mo, Na, B content, total carotenoids, TFC, vitamin C, TPC, TAC (DPPH), beta-carotene, TAC (ABTS+), sixteen phenolic acids and flavonoids were remarkably increased with the severity of drought stress. At moderate and severe drought stress conditions, the increments of all these components were more preponderant. *Trans*-cinnamic acid was newly identified phenolic acid in *A. tricolor*. Salicylic acid, vanilic acid, gallic acid, chlorogenic acid, *trans*-cinnamic acid, rutin, isoquercetin, *m*-coumaric acid and *p*-hydroxybenzoic acid were the most abundant phenolic compounds in this genotype. In *A. tricolor*, drought stress enhanced the quantitative and qualitative improvement of nutritional and bioactive compounds, phenolic acids, flavonoids and antioxidants. Hence, farmers of semi-arid and dry areas of the world could be able to grow amaranth as a substitute crop.

Four selected *A. tricolor* cultivars were grown under four irrigation regimes (25, 50, 80, and 100% field capacity) to evaluate the mechanisms of growth and physiological and biochemical responses against drought stress in randomized complete block design with three replications. Drought stress led to decrease in total biomass, specific leaf area, relative water content (RWC), photosynthetic pigments (chlorophyll a, chlorophyll b, chlorophyll ab), and soluble protein and increase in MDA, H_2O_2, EL, proline, total carotenoid, ascorbic acid, polyphenols, flavonoids, and antioxidant activity. However, responses of these parameters were differential in respect to cultivars and the degree of drought stresses. No significant difference was observed in control and LDS for most of the traits. The cultivars VA14 and VA16 were identified as more tolerant to drought and could be used for further evaluations in future breeding programs and new cultivar release programs. Positively significant correlations among MDA, H_2O_2, compatible solutes, and non-enzymatic antioxidant (proline, TPC, TFC, and TAC) suggested that compatible solutes and non-enzymatic antioxidant played vital role in detoxifying of ROS in *A. tricolor* cultivar. The increased content of ascorbic acid indicated the crucial role of the ASC–GSH cycle for scavenging ROS in A. tricolor.

The study was evaluated to explore physiological, non-enzymatic and enzymatic detoxification pathway of reactive oxygen species (ROS) in tolerance of *A. tricolor* under drought stress. The tolerant genotype VA13 exhibited lower reduction in growth, photosynthetic pigments, relative water content (RWC) and negligible increment in electrolyte leakage (EL), lower increment in proline, guaiacol peroxidase (GPOX) activity compared to sensitive genotype VA15. This genotype also had higher catalase (CAT), superoxide dismutase (SOD), remarkable and dramatic increment in ascorbate-glutathione content, ascorbate-glutathione redox and ascorbate-glutathione cycle enzymes activity compared to sensitive genotype VA15. The negligible increment of ascorbate-glutathione content, ascorbate-glutathione redox and ascorbate-glutathione cycle enzymes activities and dramatic increment in malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and EL were observed in the sensitive genotype VA15. SOD contributed superoxide radical dismutation and CAT contributed H_2O_2 detoxification in both sensitive and tolerant varieties, however, these had a great contribution in the tolerant variety. Conversely, proline and GPOX accumulation was higher in the sensitive variety compared to the tolerant variety. Increase in ascorbate-glutathione cycle enzymes activities, CAT, ascorbate-glutathione content, SOD, and ascorbate-glutathione redox clearly evident that CAT, ascorbate-glutathione cycle and SOD played a vital role in detoxification of ROS in the tolerant variety of *A. tricolor*.

Response of nutrients, dietary fiber, minerals, antioxidant phytochemicals and total antioxidant activity in three selected *A. tricolor* genotypes to varying salinity stress were investigated. The biochemical contents and antioxidant activity in *A. tricolor* leaves were significantly influenced by salt stress. Protein, ash, energy, dietary fiber, Ca, Mg, Fe, Mn, Cu, Zn, Na, beta-carotene, ascorbic acid, total polyphenol content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC) (DPPH) and total antioxidant capacity (TAC) (ABTS+) in leaves were remarkably increased at 50 mM and 100 mM NaCl concentrations. Contents of vitamins, polyphenols and flavonoids showed a good antioxidant activity due to positive and significant interrelationships with total antioxidant capacity. It revealed that *A. tricolor* can tolerate a certain level of salinity stress without compromising the nutritional quality of the final product. Taken together, our results suggest that *A. tricolor* could be a promising alternative crop for farmers, especially in salinity prone areas- in the tropical and sub-tropical regions.

A. tricolor is a unique source of betalain (betacyanin and betaxanthin), source of natural antioxidants like leaf pigments, vitamins, polyphenol, flavonoid in leafy vegetables. It had a great importance for the food industry as these compounds detoxify ROS in human and

involved in defense against several diseases. Moreover, previous literature has shown that salt stress elevated these compounds in many leafy vegetables. So, we evaluated the effect of salinity stress of these compounds in amaranth. Three selected *A. tricolor* genotypes were studied under three salinity stress to evaluate the response of these compounds. Genotype, salinity stress and genotype × salinity stress interactions significantly affected all the studied traits. A significant and remarkable increment in L, a*, b*, chroma, betacyanin, betaxanthin, betalain, total carotenoids, beta-carotene, ascorbic acid, total polyphenol content, total flavonoid content, total antioxidant capacity were observed under 50 mM and 100 mM NaCl concentrations. Bioactive leaf pigments, beta-carotene, vitamin C, phenolics and flavonoids showed good antioxidant activity due to positive and significant interrelationships with total antioxidant capacity. *A. tricolor* can tolerate salinity stress without compromising the high quality of the final product. Therefore, it could be a promising alternative crop in saline-prone areas around the globe.

A. tricolor genotype VA13 was evaluated under four salinity stress in terms of color parameters, leaf pigments, beta-carotene, vitamin C, TPC, TFC, TAC, phenolic acids and flavonoids. Salinity stress significantly increases all the studied traits. The increments of all these compounds were high under moderate and severe salinity stress compared to control condition. In this study, *trans*-cinnamic acid was newly identified phenoic acid in *A. tricolor*. Salicylic acid, vanilic acid, *trans*-cinnamic acid, gallic acid, chlorogenic acid, rutin, isoquercetin and *m*-coumaric acid were the most abundant phenolic compounds of amaranth that increased with the severity of salinity stress. *A. tricolor* leaves are good source of pigments, beta-carotene, vitamin C, bioactive compounds, phenolic acids, flavonoids and antioxidants. In salt-stressed amaranth, correlation studies revealed strong antioxidant activity of leaf pigments, beta-carotene, vitamin C, TPC, TFC. These bioactive compounds played a vital role in scavenging ROS and could be beneficial to human nutrition by serving as a good antioxidant and antiaging source in human health benefit. *A. tricolor* cultivated under salinity stress conditions can contribute a high quality of the final product in terms of leaf pigments, bioactive compounds, phenolic acids, flavonoids and antioxidants. It can be a promising alternative crop in saline-prone areas.

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