Determination of Dark-germination Properties during Seed Development of Nicotiana tabacum L.

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Determination of Dark-germination Properties during Seed Development of *Nicotiana tabacum* L.*

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SUMMARY

Two substances inhibiting the nucleic acid metabolism, actimnomycin D and puromycin, were injected into capsules of *Nicotiana tabacum* L. cv. Xanthi during seed development. The dark-germination percentage of seed from capsules dereased to 64.7% in the case of actinomycin D injection caused the highest inhibition of dark-germination (21.3%) at 7 days after self-pollination. Viability of seeds did not differ among treatments. The regulatory action of dark-germination genes was discussed.

INTRODUCTION

It has been recognized that exposure to light is required for tobacco seed germination, but some varieties of tobacco germinate in darkness[1]. They are called the dark-germinating varieties. The inheritance of dark-germinating property of tobacco seeds was explained by the double recessive gene system[2]. The phytochrome of tobacco seeds affects the germination in darkness.

The present work seeks to ascertain the genetical nature of the mechanism in the determination of dark-germination properties during seed development.

MATERIALS AND METHODS

Xanthi, a dark-germinating variety was planted in the clay pot in mid May. Twenty plants were grown in a greenhouse under natural light and temperature conditions in the summer season.

The number of days after artificial self-pollination was used as the index of seed development in the capsules. Two inhibiting substances of the nucleic acid metabolism, actinomycin D (Nakor Chemicals Ltd, Israel) and puromycin (Nakor chemicals Ltd, Israel) were used at a concentration of 100 ppm. Fifty μl of each substance or 50 μl of distilled water as control were injected by a micro -syringe (Ito MS-100) into three capsules of different plants per treatment, respectively. Each injection treatment of capsules was performed at 3, 5, 7 and 10 days after self-pollination.

At 30 days after self-pollination, the capsule-injected seeds were harvested from mother plants. The seeds were isolated from each capsule, air-dried and kept in paper bag at room temperature. Two dishes per injected capsules were used in the germination tests. One hundred seeds from a capsule were sown on two layers of Toyo No. 1 filter paper moistedmed with 2 ml of distilled water in a petri dish. Then they were wrapped with a light-tight-proof paper and placed in a temperature -controlled chamber at 20° C for 7 days; the viabilty of seeds was evaluated by a succession of germination tests for 7 days under light condition. The percentage of germination was determined at

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7 days after sowing and expressed as the mean value of the three capsules. Germination percentage was transformed into angle scale prior to analysis.

RESULTS AND DISCUSSION

We obtained mature seeds from all treated caples which were sufficient for germination tests, although a part of some capsules was partially sterile after treatments. Table 1 describes the results of dark-germination tests. The evaluated viabilty of seeds did not differ among three treatments (Table 1). The average germination percentage was 92.8%. The seeds from the water-injected capsules at any developmental stage of seeds showed a high germination percentage in darkness (Table 1). The average of germination percentage was 83.7%. At 3 days after self-pollination, there were differences in the results between actinomycin D injection treatment and water injection treatment. The effects of actinomycin D injection became clear at 5 days after self-pollination. The germination of seeds from actinomycin D-injected capsules at 5 days after self-pollination decreased to 64.7%.

As ripening progressed, the germination percentage of actinomycin D-treated seeds increased and their value at 10 days after self-pollinationid not differ from germination percentage of seeds from water-injected capsules Actinomycin D was found to be inhibitive for the early stage of seed development (Table 1).

Puromycin-injection was most effective at 7 days after self-pollination. The dark-germination percentage was 62.3%. The effects of puromycin injection became slightly clearer at 5 days after self-pollnation. The germination percentage decreased by 9.7% compared with the germination percentage of seeds at 3 days after self-pollination. At 10 days after self-pollination, there were no effects of actinomycin D injection and puromycin injection on the dark-germination. The dark-germination percentage increased to more than 80%.

It was indicated that both actinomycin D injection and puromycin injection inhibited the process of determination of dark-germination ability during seed development. Dark-germination ability was brought about gene action during seed ripening. But expression of dark-germination gene did not result because of the inhibiting action of actinomycin D and puromycin at the early stage of seed development. Therefore, the dark-germination percentage showed a decline. Because the viability of

Table 1. Dark-germination and viability of Xanthi seeds from capsules injected inhibiting substances of nucleic acid metabolism at several stages of seed development.

Substance	Stage of seed development (Days after artificial self-pollination)			
	3	5	7	10
a) Dark-germination (%)				
Water	82.0_a	84.3_a	83.6_a	84.6_a
Actinomycin D (100 ppm)	76.0_b	64.7_c	79.0_b	81.7_a
Puromycin (100 ppm)	80.0_a	75.3_b	62.3_c	81.3_a
b) Viability				
Water	91.7_a	93.7_a	93.1_a	94.2_a
Actinomycin D (100 ppm)	92.4_a	92.6_a	92.7_a	92.3_a
Puromycin (100 ppm)	93.5_a	92.7_a	92.7_a	92.1_a

Note : Means followed by the same letter are not significantly different at P=0.05 level by Duncan's multiple range test.

seeds did not differ among treatments, it can be said that the treatments by two substances influenced the dark-germination property.

Actinomycin D is a strong inhibitor of the DNA-dependent RNA-polimerase reaction. This explained the specific suppression of mRNA synthesis by actinomycin D. The genetic information is transferred by the aid of mRNA. The information contained in the nucleotide code of mRNA was transferred to the amino acid sequence of protein that was formed. The addition of puromycin to protein synthesis leads, not only to strong inhibition, but also to the liberation of small peptide fragments[3]. Protein synthesis is one of the essential steps in seed formation. These treatments of two inhibiting substances partially induced the light requirement for seed germination.

The realtionship of phytochrome action and nucleic acid metabolism in seed formation or germination is not obvious. It has been demomstrated that phytochrome regulated the transcription of specific genes[4]. Phtochrome is involved in the light regulation of a number of plant responses. Phytochrome of seeds light-requiring variety can be manipulated with light and hydration[5,6]. Dark-germination genes were differentiated from light sensitive-mechanism[7]. Dark-germination of Xanthi and germination of phytochrome-manipulated light-requiring seeds were highly similar phenomena, but they would be difficult to explain by the same mechanism.

We have discussed how dark-germination gene was concerned with determination of the Pfr/Pr ratio of phytochrome when seeds were matured. Since neither actinomycin D nor puromucin influenced the viability of seeds obtained, the determination of Pfr/Pr ratio of phytochrome determined the dark-germination property.

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タバコ種子の発育期間における暗発芽性の決定

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核酸代謝を阻害するアクチノマイシンDとピュロマイシンをタバコの一品種 Xanthi の種子発育中の蒴にそれぞれ注射した。得られた種子の暗発芽率は対照区（水注射）の82.0～84.6%に対して，アクチノマイシンDの場合，受粉後5日目で64.7%，ピュロマイシンの場合，受粉後7日目で，62.3%であった。しかし，種子の活力は影響を受けなかった。受粉後一週間以内の種子発育期間中の核酸代謝阻害物質の投与はタバコ種子暗発芽性に影響を与える。

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