In vitro growth of Japanese persimmon (Diospyros kaki Thunb.) seedling plants.(1)

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In vitro growth of Japanese persimmon (*Diospyros kaki* Thunb.) seedling plants. (1)

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SUMMARY

The Japanese persimmon cannot propagate by cutting because of low rooting potential, so the rootstocks have been propagated by seeding. The seedling stock, because of heterozygosity, has various gene properties in root growth; consequently, the growth of the scion, that is, tree growth which is affected by root growth of the seedling stock, has been varied greatly. This variation of tree growth has made it difficult to obtain standardization of commercial orchard management, and the multiplication of clonal rootstocks has therefore been desired. The present paper describes the establishment and the growth of clonal seedling plant *in vitro*. The aseptic seeding and the unsterilized embryo culture have not been established due to contamination by fungi. Embryos, however, which were re-sterilized by 70% ethanol grew normally, and the contamination percentage was only 1%. Shoot elongation from re-sterilized embryo was enhanced by GA_3 but this effectiveness of GA_3 was inhibited by an increased BAP concentration. Additional NAA intensely inhibited shoot elongation. Root elongation was inhibited at high concentrations of NAA over 1μM. A supplement of BAP or GA_3 has no effect on rooting. Five lines were selected from among a group of 'Fuyu' seedlings for shoot elongation.

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INTRODUCTION

Since Japanese persimmons have a low rooting potential[1], the commercial varieties have been propagated not by cutting, but by grafting into seedlings. The seedling, because of heterozygosity, has various gene properties in root growth or production of plant growth regulators at root and so on. Thus, the root growth of seedling is diverse. The growth of scions, that is, tree growth is, affected by this root growth of seedling stock and is therefore diverse. This multiformity of tree growth in orchards causes a difficulty in standardization of commercial orchard management, so a multiplication of clonal rootstocks has therefore been desired especially for Japanese persimmons. Recently, tissue culture techniques have been rapidly developed, and the clonal rootstocks were micropropagated in apple[2]. In the commercial variety of the Japanese persimmon, shoot tip culture has been accomplished by Sugiura et al.[3] and the authors[4], and this culture has the probability of clonal rootstock multiplication.

In vitro seedling plants are able to grow generally by procedure of shoot tip culture or aseptic seeding. Some varieties of Japanese persimmon, however, did not accomplish the proliferation by

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shoot tip culture[5]. The present study describes the establishment of seedling plants *in vitro* and the growth of these seedling plants.

MATERIALS AND METHODS

1. Establishment of seedling plant *in vitro*.

The materials were the Japanese persimmon (*Diospyros kaki* Thunb.) cvs 'Fuyu' and 'Zenji-maru' seeds stored at low temperature (5℃) for sixty days.

(1) Aseptic seeding.

The seeds were sterilized by immersion for 15 min in 1% NaClO and then were placed on a culture medium after three rinses in sterilized distilled water. The basal medium (1/2N−MS) was that of Murashige and Skoog (1962)[6] of which the level of nitrogen was reduced to half strength and contained 3% sucrose and 0.7% agar. Plant growth regulators were free at all media. The pH of the media was adjusted to 5.7.

(2) Embryo culture.

The seeds after preparation as shown in Fig. 1 were sterilized by the same procedures as aseptic seeding, and then the embryos were taken out from seeds in the aseptic condition. The embryos were divided between two treatments; one was re-sterilized by 70% ethanol for 30 sec and then rinsed three times by sterilized water, whereas the other was not sterilized. The basal media (1/2N−MS) were the same as for aseptic seeding except they contained gibberellin A_3 (GA_3), 6-benzylaminopurine (BAP) and α-naphthaleneacetic acid (NAA).

2. Growth of seedling plant during subculture.

The explant was a single node of 'Fuyu' and 'Zenji-maru' seedling plants which was established by re-sterilized embryo culture *in vitro*. The basal media (1/2N−MS) contained zeatin 10^{-5}M.

All cultures were kept under 3000lx of 16-hour illumination at 25℃ for six weeks.

RESULTS

1. Establishment of seedling plant *in vitro*.

The aseptic seeding and the unsterilized embryo culture have not been established due to contamination by fungi (Table 1). Embryos, however, which were re-sterilized by 70% ethanol after an immersion of seeds for 15 min in 1% NaClO, grew normally and the contamination percentage was only 1%.

Effect of growth regulators on the re-sterilized embryo growth was shown in Fig. 2−4. In GA_3, BAP and NAA free, shoot elongation was not recognized. GA_3 enhanced shoot elongation appreciably, but this effectiveness of GA_3 was inhibited by an increasing BAP concentretion (Fig. 2).

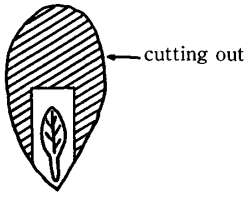

Fig. 1. Preparation of seed before sterilization.

Table 1. Contamination percentage by fungi using several culture methods.

colture method	contamination percentage
aseptic seedling	100
embryo culture	
not sterilize	91
re-sterilize by 90% ethanol	1

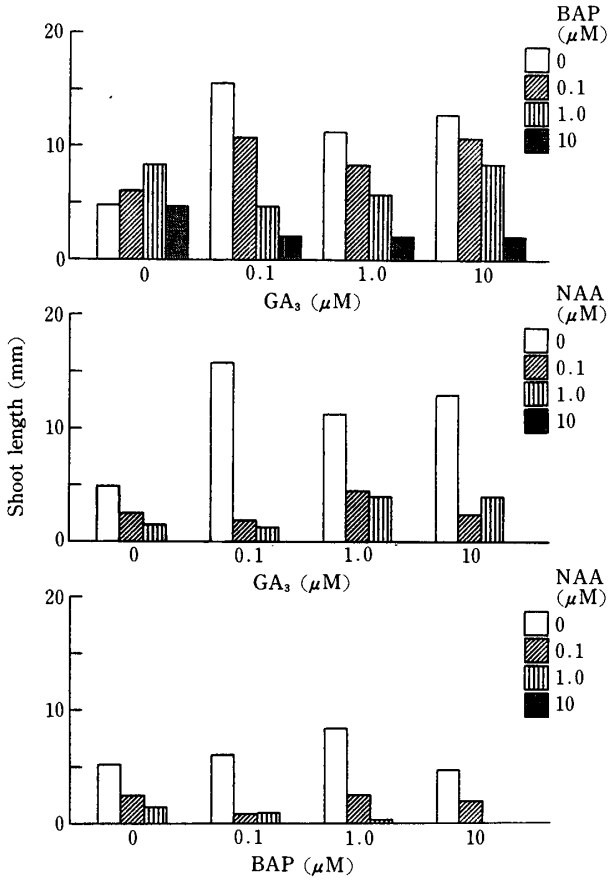

Fig. 2. Effect of growth regulators on shoot elongation from embryo (*Diospyros kaki* Thunb. cv Fuyu).

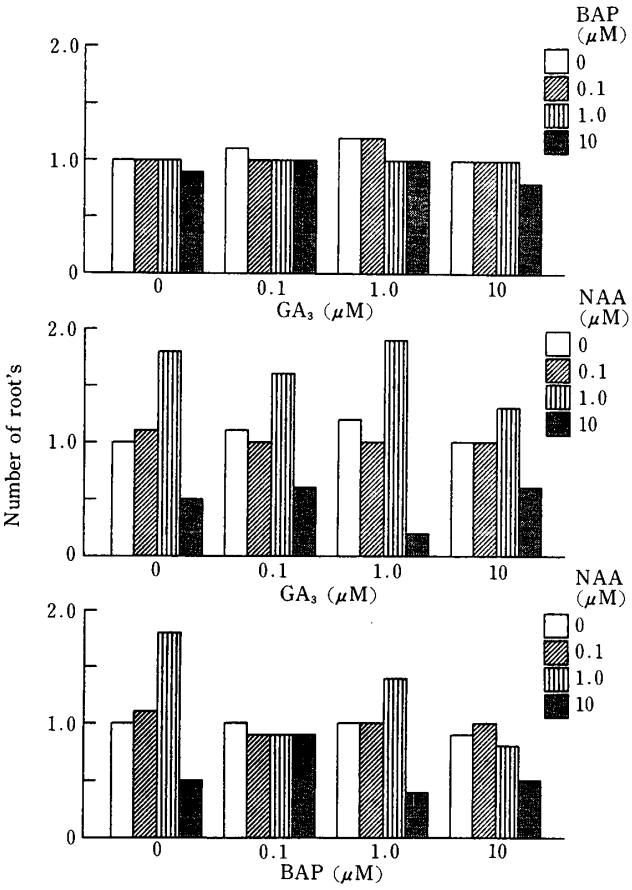

Fig. 3. Effect of growth regulators on root differentiation from embryo (*Diospyros kaki* Thunb. cv Fuyu).

Additional NAA greatly inhibited shoot elongation, especially at a high concentration of more than 1μM (Fig. 2). As recognized above, the better shoot elongation was obtained on the media in addition to GA_3 alone at the range of 0.1 and 10μM.

No explants initiated a new root because the root was only one or so, except for NAA 1 and 10μM (Fig. 3). NAA at 1μM initiated multiple roots from one embryo, since the root number at NAA 1μM was 1.8 as an average (Fig. 3). The high concentration of NAA (10μM) inhibited the original root elongation of embryos (Fig. 3).

Root elongation was inhibited at high concentrations of NAA over 1μM. Especially in NAA 10μM the root lenght was under 5mm (Fig. 4). A supplement of BAP or GA_3 is not effective.

2. Growth of seedling plant during subculture.

Growth of a group of 'Fuyu' seedling plants was superior to that of 'Zenji-maru' seedlings (Fig. 5). At the present time, five lines (from dwarfing to vigorating) were selected from among a group of 'Fuyu' seedlings for shoot elongation as shown in Fig. 6.

DISCUSSION

In many plants, *in vitro* establishment of seedling plants has been made through aseptic seeding. In the Japanese persimmon, however, no aseptic seedling plants could not be obtained either by aseptic seeding or normal embroy culture (not sterilized embryo culture) because of contamination by fungi, and only a resterilized embryo culture was useful to yield aseptic seedling plants. Although embryos

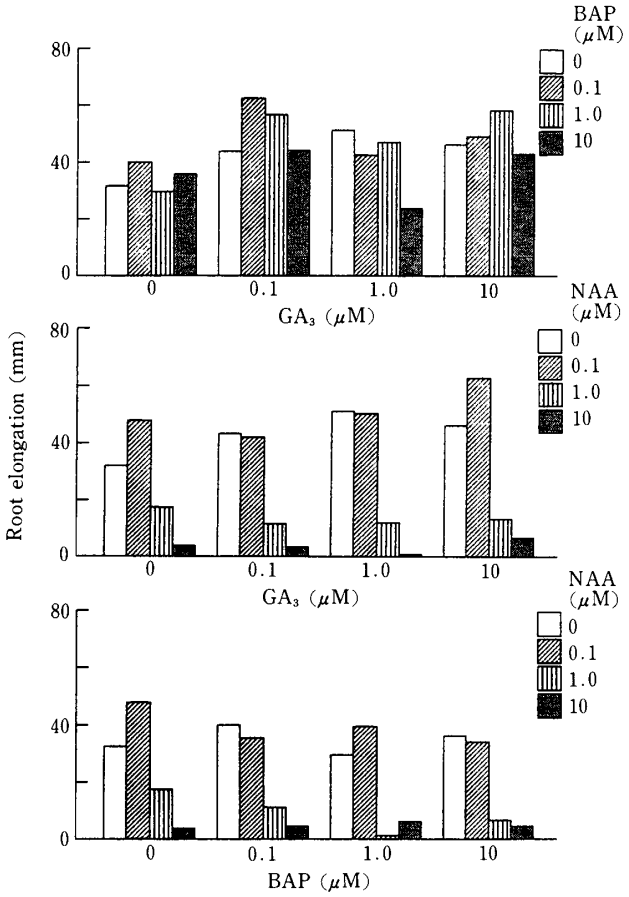

Fig. 4 Effect of growth regulators on root elongation from embryo (*Diospyros kaki* Thunb. cv Fuyu).

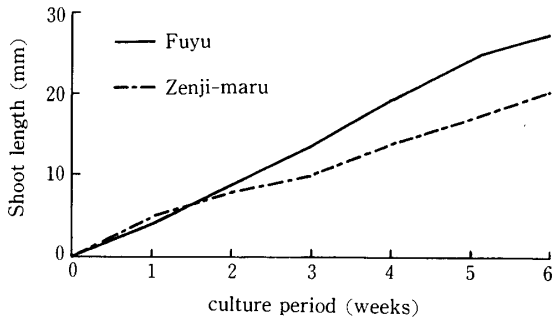

Fig. 5 Shoot growth of seeding plants from Fuyu and Zenji-maru during subculture.

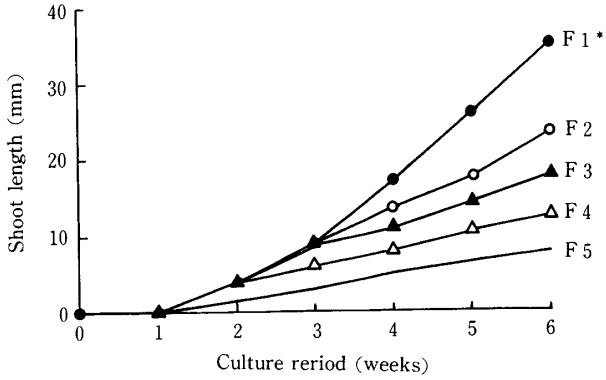

Fig. 6 Growth of several lines from Fuyu seedlings.
* *Line name* Growth of F3 is the same as the growth in shoot tip culture of Fuyu.

in seed are generally in aseptic condition, it seems that the embryo in the Japanese persimmon is contaminated by fungi growing though a large micropyle into seed. The re-sterilizing of embryo is therefore indispensable to obtain aseptic seedling plants.

GA_3 accelerated shoot growth from embryo, which BAP interfered with the effectiveness of GA_3 which has germination efficiency (Fig. 2). It was known in shoot tip culture of Japanese persimmon that BAP accelerated shoot growth in low concentrations whereas high concentrations of BAP inhibited it[3]. The reduction of shoot growth by BAP in this experiment, therefore, may result from an excess of cytokinin, that is, an addition of cytokinin activity synthesized at radicle to BAP in medium. No shoot elongated on the media with NAA. This corresponded with results in apple[7] that NAA reduced growth activity of shoot apex.

Root elongation is necessary for shoot growth. NAA in 1μM promoted root induction but inhibited root elongation. Root elongation accelerated in little (0.1μM) or no concentration of NAA. We therefore considered that the medium with little or no concentration of NAA was adequate for root elongation from embryo.

As seen from the foregoing, the re-sterilized embryo must be cultured in the medium with GA_3 of only 0.1 or 1μM.

Recently, there has been wide spread growth of orchard management keeping tree height low by dwarfing rootstocks in apples, cherries and so on. This dwarfing controlled management has made for a reduction of labor and the increase of yield per unit area. With the Japanese persimmon, no

dwarfing controlled management has been adopted. The result has been in no propagation of dwarfing rootstocks due to the difficulty of cutting and less search for dwarfing lines because of the little geographical distribution of *Diospyros*. The plantlet with a little shoot elongation in this experiment was selected from subculturing five lines in 'Fuyu' and was proliferated by single node cutting. In the near future, we will research the possible correlation between shoot elongation *in vitro* with that after potting.

ACKNOWLEDGMENT

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カキ（*Diospyros Kaki* Thunb.）の実生個体の *in vitro* における生長

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要　約

カキは発根能が低く，挿し木繁殖が困難であるため，台木の繁殖は実生を用いている。実生台木は遺伝的性質が各々異なりその生育が一様ではないため，栽培圃場では樹勢にバラツキが見られ，圃場管理に大きな支障を来している。本研究では組織培養による均一台木の大量増殖を目的として行った。無菌播種及び通常の胚培養は雑菌汚染により定着しなかった。しかし種子を殺菌後胚を摘出し，エタノールで胚を再度殺菌したものの雑菌汚染率は1％であった。摘出後再度殺菌した胚のシュートの伸長はGA_3で促進されたが，そのGA_3のシュート伸長促進効果はBAPにより打ち消された。NAAは著しくシュートの伸長を阻害した。根の伸長は，NAA無添加もしくは0.1μMの低濃度において促進され，GA_3及びBAPはほとんど作用を示さなかった。冨有の実生を系統的に分別したところ，葉形，シュートの伸長程度から5系統の植物体が得られた。