

Effect of Sugars on Rooting of Shoots of Japanese Persimmon Propagated *in vitro*

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Abstract

The effect of different concentrations of sucrose, glucose and fructose on *in vitro* rooting of shoots of Japanese persimmon that had been induced from selected rootstocks was studied. When the shoots of Japanese persimmon strain "No.3" were cultured on autoclaved half-strength Murashige and Skoog medium (MS medium) containing 0.2M fructose, roots were induced in 94% of the shoots after 45 days of culture whereas only 30% of rooting was obtained on media containing the same concentration of sucrose or glucose. However, filter-sterilized fructose had no stimulative effect on rooting. The autoclaved medium containing 0.2M fructose showed consistently high percentages of rooting (80%<) of the shoots for 9 rootstock strains tested, whereas only 3 strains responded to the culture on medium containing 0.1M sucrose following 250 mg/l IBA pretreatment.

1. Introduction

Rootstocks of Japanese persimmon (*Diospyros kaki* Thunb.) have usually been multiplied by seedling because they are difficult to propagate by cuttings due to poor rooting ability. Consequently, tree growth and fruit productivity of a cultivar are variable in the orchard because of the heterozygous nature of the rootstocks. Therefore, it is necessary to establish an efficient method of clonal propagation of rootstocks to improve the uniforming of the orchard. Clonal propagation by micropropagation has been reported in some fruit trees which are difficult to propagate by cuttings, such as apple (Jones *et al.* 1977; James and Thurbon 1979, 1981), peach (Miller *et al.* 1982) and plum (Jones and Hopgood, 1979). Although there are also some reports on micropropagation in Japanese persimmon (Cooper and Cohen, 1985; Sugiura *et al.* 1986; Fukui *et al.* 1988; Murayama *et al.* 1989; Fukui *et al.* 1989, 1990), root induction from *in vitro* shoots of Japanese persimmon still remains the limiting step in many cultivars even with the application of auxin treatment (Murayama *et al.* 1989; Fukui *et al.* 1992).

In the present study, the author reports the importance of sugars on *in vitro* rooting of the shoots of rootstock Japanese persimmon which is difficult to root.

2. Materials and methods

2.1 Plant materials

Nine strains of Japanese persimmon, which had been selected in a breeding program for rootstock at Deciduous Fruit Tree Branch, Shizuoka Prefectural Citrus Experiment Station (Hamamatsu City JAPAN), were used. Growth habit and fruit productivity of these nine strains are summarized in **Table 1**.

Table 1. Some characteristics of the rootstock strains of Japanese persimmon used in the present study

strain ^z	canopy volume (m ³) ^y	fruit productivity (kg) ^x
No.3	17.7	44.6
S15	6.8	57.0
S16	12.9	93.8
S22	23.5	119.6
S24	21.1	124.3
S29	23.8	138.4
S56	38.5	181.3
S8	69.6	116.1
S9	58.1	76.3

^z: The scion used 'Maekawajiro'

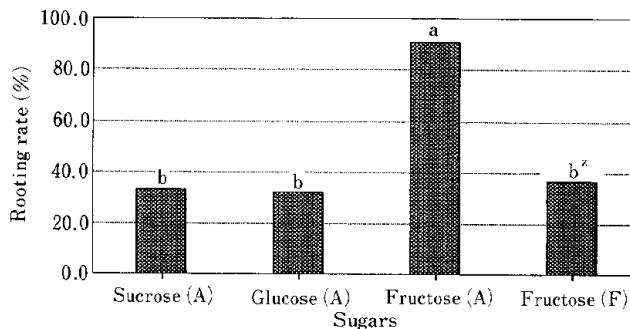
^y: canopy volume was determined in 1987.

^x: fruit productivity was expressed as the summary of the fruit yield from 1982 to 1987.

Table 2. Effect of sugars on rooting of *in vitro* shoots of No.3 strain of Japanese persimmon

Sugars	conc. (M)	Rooting (%)	Number of roots	Root length (mm)
Sucrose	0.05	20.0cd ^z	2.5ab	4.3d
	0.1	28.9c	1.4b	22.0bc
	0.2	33.3c	2.4ab	10.6c
Glucose	0.05	8.9bc	1.7b	4.7d
	0.1	37.8bc	2.3b	25.1bc
	0.2	32.3c	1.0b	11.9c
Fructose	0.05	53.3b	1.4b	13.5c
	0.1	77.7a	3.2ab	356.9b
	0.2	94.4a	3.9ab	55.1a

^z: Separation by Duncan's multiple range test at $p=0.05$
All media were autoclaved.

**Fig. 1** Effect of sugars and autoclaving on rooting of shoots of No.3 strain

(A):media containing sugars were autoclaved.

(F):filter-sterilized fructose was added to the medium after autoclaving.

concentration of sucrose was 0.1M, whereas those of glucose and fructose (A)and (F)were 0.2M.

Data were taken after 45 days of culture.

^z:Separation by Duncan's multiple range test, at $p=0.05$

2.2 Shoot tip culture and subculture of shoots *in vitro*

For each of the 9 rootstock strains listed in **Table 1**, the roots of a plant grown in the field was injured near the trunk using a saw or an ax to induce adventitious shoots in the wound.

Shoot tips (2 mm long) were excised from axillary buds of the shoots which emerged and were cultured aseptically on the basal medium, modified Murashige and Skoog (MS)(Murashige and Skoog, 1962) (1/2 nitrogen) medium, containing 2mg l⁻¹ zeatin and 30g l⁻¹ sucrose (Fukui *et al.* 1989, 1990). The pH of the medium was adjusted to 5.8 with 0.1N NaOH and solidified with 7g l⁻¹ agar(Wako pure chemical industries, Ltd. for plant tissue culture medium), before autoclaving at 120 °C , 1.2kg/cm²

for 15 minutes. Cultures were kept at 25 °C with a 16 hr photoperiod at 4000 lux.

Elongated shoots were cut into nodal segments each with one axillary bud *in vitro*. The four nodal segments were cultured in a glass tube (60 mm in diameter, 150 mm long) containing the same modified MS medium supplemented with 0.5 mg l⁻¹ zeatin to reproduce shoots (kagami *et al.* 1995). The tubes were capped with plastic caps. The shoots were subcultured every 2 months by repeating the nodal segment culture on a fresh medium for 2 years. The shoots which had 2-4 leaves of 2-3 cm long, obtained 40-50 days after subculture were used for rooting experiments.

2.3 Induction of rooting

(1)Preference of sugars on rooting

The shoots (2-3 cm) of the strain "No.3"were cultured on the half-strength modified MS medium with sucrose, glucose or fructose (Wako pure chemical industries, Ltd. special) at concentrations of 0.05, 0.1 and 0.2 M. Number of rooted shoots, roots per rooted shoot and length of roots per shoot were recorded after 45 days of culture and these values were compared among all the treatments by Duncan's multiple range test ($p=0.05$).

(2)Comparison in rooting percentages between the autoclaved and filter-sterilized fructose medium

Comparison in rooting were made after 45 days of culture among the autoclaved medium containing 0.1 M sucrose, 0.2 M glucose or 0.2 M fructose and that with filter-sterilized 0.2 M fructose. Filter-sterilized 0.2 M fructose medium was made by mixed autoclaved 1/2 modified MS and 0.8 M fructose which was sterilized using nitro-cellulose filter of 0.2 micrometer pore size. The data on rooting of the shoots on these media were recorded after 45 days of culture. The differences in the percentage of rooted explants were compared among all the treatments by Duncan's multiple range test ($p=0.05$).

(3) Comparison of rooting frequencies among the 9 strains under 2 root inducing methods

Efficiency of root induction was compared between the autoclaved medium containing 0.1 M sucrose and that containing 0.2 M fructose using 9 strains of rootstocks. In the culture with sucrose-containing medium, the basal part of the shoots was dipped in 250 mg l⁻¹ indole-3-butyric acid (IBA) solution for 10 sec. and then cultured on the basal medium with 0.1 M sucrose, whereas the shoots for the culture on the basal medium containing fructose were not treated with IBA. The rooting frequencies for 9 strains were also recorded after 45 days of culture. The differences in rooting were compared

by Duncan's multiple range test ($p=0.05$) among the 9 rootstocks in each of the 2 root inducing methods.

Throughout the rooting experiments, glass test tubes (25 mm diameter, 150 mm depth), each containing 15 ml of medium which was solidified with 2g l⁻¹ gellan gum (wako pure chemical industries, Ltd. for plant tissue culture). Culture condition such as temperature, light, photoperiod, sterilized method and pH of the medium were same with shoot proliferation. Three replications of the treatments were made for each treatment and thirty shoots were used for each treatment.

Rooted shoots were potted in autoclaved vermiculite and then kept in an incubator to acclimatize for 2 months at 25 °C. Then they were transferred to a greenhouse.

4. Result and Discussion

In the previous study on apple, rooting of shoots in a rootstock strain was stimulated by sucrose rather than filter-sterilized fructose to test the effect of sugars and sugar alcohol (Chong and Pua, 1985). In cork oak (*Quercus suber* L.), the medium containing autoclaved fructose had deleterious effects on the root induction from shoots (Romeo *et al.* 1995). In the present study, however, 0.2 M autoclaved fructose showed the highest values for frequency in rooting (94.4%), number of roots per rooted shoot (3.9) and length of roots per shoot (55 mm) (Table 2). The frequencies of rooting in medium with sucrose or glucose were 1/3 of those with fructose. The frequency of rooting in medium containing autoclaved fructose was as 3 times high as that in medium containing filter-sterilized fructose. No significant difference was found between the autoclaved medium containing sucrose and that with glucose (Fig 1).

Coloring of the medium to light brown and acidification have been observed after autoclaving the mixture of medium nutrients and fructose (Owen *et al.*, 1991; Durart and Wulf, 1993). In the present study, the medium with fructose was turned light brown and showed a lower pH of 4.0-4.2 after autoclaving. It was reported that fructose was degraded by autoclaving and acidic condition and then frufural and hydroxymethylfrufural were formed (Shaw *et al.*, 1967; Durart and Wulf, 1993).

Thus, it is possible to postulate that root induction from *in vitro* shoot of the rootstocks of Japanese persimmon by autoclaved fructose was stimulated by the compound(s) which might be created by degradation of fructose.

Auxins were commonly used for root induction from *in vitro* shoots of fruit trees. In Japanese

persimmon, shoots were usually rooted by briefly dipping in 250 mg l⁻¹ IBA solution or 0.4 % α -naphthyl-acetamide (NAM) powder before culturing on modified MS medium with 30 g l⁻¹ sucrose (Cooper and Cohen, 1985; Sugiura *et al.* 1986; Fukui *et al.* 1988; Murayama *et al.* 1989).

In the present study, however, only 3 strains showed more than 80 % shoots that rooted and 6 strains showed less than 60 % with the treatment of IBA. Similar genotypic differences were previously observed in Japanese persimmon with the root inducing method using sucrose medium in combination with auxin pretreatment (Fukui *et al.* 1988). Among the 95 varieties of Japanese persimmon tested *in vitro*, 25 showed no rooting, 31 less than 30%, 23 40-60%, and 16 more than 70% rooting of the shoots when they were tested with NAM (α -naphthyl-acetamide) powder. Murayama *et al.* (1989) reported that rooting frequencies of Jiro, Aizumishirazu, E-goshyo, Kurogaki were 80, 74, 44 and 8%, respectively, by dipping in IBA solution. In the present study, on medium containing fructose was used, more than 80% rooting frequency was obtained on medium containing fructose in all 9 strains tested (Fig. 2). Thus, the autoclaved medium containing fructose stimulated rooting of rootstocks of Japanese persimmon irrespective of genotypic difference.

These results show that some compound(s) produced by autoclaving the medium containing fructose may as a stimulative factor(s) for the adventitious root formation from Japanese persimmon shoots.

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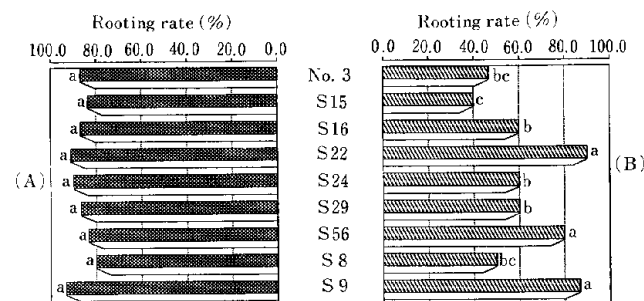


Fig. 2 Difference in rooting among the 9 rootstock strains on the medium containing 0.2M fructose (A) and that containing 0.1M sucrose after dipping in 250mg l⁻¹ IBA solution (B). Data were taken after 45 days of culture.

∴ Separation by Duncan's multiple range test, at $p=0.05$

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