Single-node Stem Cuttings from Root Suckers to Propagate a Potentially Dwarfing Rootstock for Japanese Persimmon

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ADDITIONAL INDEX WORDS. *Diospyros kaki*, juvenility

SUMMARY. A potentially dwarfing rootstock for Japanese persimmon (*Diospyros kaki* L.) was propagated by single-node stem cuttings taken from root suckers. When a mature tree was cut down at ground level and part of the roots was exposed to the air, numerous suckers formed on the exposed parts of the roots. Single-node stem cuttings 3 to 4 cm (1.2 to 1.6 inches) long survived and rooted better than 10-cm (3.9-inch) and 25-cm (9.8-inch) leafy stem cuttings with several buds. Dipping cuttings in 3000 mg·L⁻¹ (ppm) IBA for 5 s or in 25 mg·L⁻¹ IBA for 24 h resulted in similar rooting. Most of the single-node stem cuttings taken in late-June and July survived and rooted well, whereas those prepared in late August rooted poorly and few survived. The survival and rooting percentages were unaffected by the position on the suckers (top vs. base) from which cuttings were taken. High relative

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humidity in the propagation frame appeared to enhance survival and rooting. This clonal propagation method will make a rapid multiplication of Japanese persimmon, a difficult-to-root species, possible. Chemical name used: indole-3-butyric acid (IBA).

Japanese persimmon tends to become a large tree, and growers often encounter difficulties in orchard management (Tao and Sugiura, 1992). So far, dwarfing rootstocks have been unavailable for persimmons except for diamond-leafed persimmon (*Diospyros rhombifolia* Hemsl.), but 'Fuyu' trees grafted on diamond-leafed persimmon grew poorly and the yield was insufficient (Yamada et al., 1997). Decline of Japanese persimmon trees grafted on diamond-leafed persimmon has been observed, likely due to a graft incompatibility (Yamada et al., 1997).

Occasionally dwarfed persimmon trees grafted on Japanese persimmon seedlings are found in commercial orchards, and Kimura et al. (1985) assumed that such rootstocks might have a genotypic dwarfing ability. As propagation of Japanese persimmon by cuttings is very difficult (Tao and Sugiura, 1992), clonal propagation by micropropagation of potentially dwarfing rootstocks has been tried. Several researchers have succeeded in micropropagating such rootstocks (Kagami et al., 1995). Negative characteristics, however, may be induced in these plants as is sometimes seen with apple (Jones and Hadlow, 1989). Micropropagated and own-rooted 'Nishimurawase' Japanese persimmon trees showed more vigorous vegetative growth than those grafted on seedlings (Tetsumura et al., 1999). Although nursery performance of 'Cox' apple trees (*Malus × domestica* Borkh.) on micropropagated Malling 9 dwarfing rootstocks was undesirable, Jones and Webster (1993) overcame this problem using rootstocks from improved conventional propagation from micropropagated plants. Therefore, micropropagated dwarfing rootstocks for persimmons might be undesirable, and development of alternative propagation methods for the dwarfing rootstocks is needed.

Fordham (1969) propagated several difficult-to-root tree species by cut-

tings of shoots arising from root pieces. Robinson and Schwabe (1977) indicated that adventitious shoots from the root cuttings of 'Lord Lamborne' apple clones had a high rooting potential. In vitro experiments with Japanese persimmon showed that the shoots differentiated from root pieces (Tetsumura and Yukinaga, 1996) rooted better than those originating from shoot tips (Tetsumura and Yukinaga, 2000). Hence, cuttings from adventitious shoots formed on in vivo roots of Japanese persimmon may root easily and

facilitate its clonal propagation. The objective of the present study was to develop a vegetative propagation method for potentially dwarfing rootstocks for Japanese persimmon by using adventitious shoots arising from roots.

Materials and methods

CUTTING SOURCE. A mature dwarfed 'Hiratanenashi' tree grafted on a persimmon seedling (parents unknown) and planted in 1963 at an orchard of the Experimental Farm of Kyoto University was used for this study. In March 1996,

Fig. 1. Propagation of the persimmon rootstock by single-node stem cuttings from root suckers and production of the grafted nursery plants. (A) Root suckers sprouting and elongating late in May. (B) A well-rooted single-node stem cutting 60 d after the rooting treatment. Ruler = 19 cm (7.5 inches). (C) Elongating shoots from 'Hiratanenashi' scion wood on the nursery plants 60 d after grafting. Ruler = 30 cm (11.8 inches).

the top of the tree, including the root-stock shank, was cut off just above the ground level. Then, the surface soil about 1 m² (10.8 ft²) around the stump and 30 cm (11.8 inches) in depth was removed, and part of the root was exposed to the air to stimulate differentiation of adventitious shoots (root suckers). Before being felled, the tree was 3.7 m (12.1 ft) in height, 37.5 m² (404 ft²) in coverage, and 71 m³ (2500 ft³) in volume, whereas the average dimensions of 30- to 40-year-old normalized 'Hiratanenashi' trees in the same orchard were 5.3 m (17.4 ft), 116.0 m² (1250 ft²), and 335 m³ (11800 ft³), respectively. The average annual yield for 1993 to 1995 was 58 kg (128 lb) per tree, 1.55 kg·m⁻² (0.32 lb/ft²), and 0.82 kg·m⁻³ (0.052 lb/ft³) for the dwarfed tree, and 145 kg (320 lb) per tree, 1.25 kg·m⁻² (0.26 lb/ft²), and 0.43 kg·m⁻³ (0.027 lb/ft³) for the normal-sized trees.

EXPERIMENT 1 (CUTTING LENGTH AND METHODS OF ROOTING TREATMENT). The experiments were conducted in 1996. On 1 July, vigorously growing suckers were harvested leaving 30-cm proximal ends, and the uppermost soft parts were discarded. Three types of cuttings were prepared with regard to position on the suckers: 1) 25-cm-long cuttings with 5 to 7 buds and two halved terminal leaves; 2) 10-cm-long cuttings with 3 to 4 buds and two halved terminal leaves; 3) 3- to 4-cm-long stem cuttings with one bud and one leaf (single-node stem cuttings). The bases of 30 cuttings of each type were dipped for 5 sec in a 3000 mg·L⁻¹ IBA solution with 50% (v/v) ethanol (quick dip), whereas the bases of another 30 cuttings of each type were kept for 24 h in an aqueous solution of 25 mg·L⁻¹ IBA. After the rooting treatments, cuttings were vertically stuck in vermiculite (small grain type) in 20-L, 17-cm-deep (6.7-inch-deep) plastic baskets. Twenty cuttings were stuck per basket, and ten cuttings per plot were stuck in a completely randomized experimental design. The baskets with cuttings were placed under a shade net with vaporized aluminum in a propagation frame covered with plastic film that opened at the sides, which provided them with 24% irradiance (photosynthetically active radiation) of the ambient sunlight. The propagation frame was intermittently misted using a screen-balance switch (Kodama Automatic Watering Ind. Co., Osaka, Japan), which turned the mist sprayer on when the

netted screen became dry. The survival and rooting percentages and the number of roots per rooted cutting were recorded 90 days after the rooting treatment. Percentage data were subjected to trend analysis of effects of cutting length through linear and quadratic relationships. The other data were subjected to analysis of variance (ANOVA).

EXPERIMENT 2 (TIME OF CUTTING COLLECTION AND POSITION OF CUTTINGS ON THE SUCKERS). The experiments were conducted in 1997, 1998 and 1999. On 23 June, 24 July, and 20 Aug., suckers longer than 130 cm (4.3 ft) were harvested leaving the proximal 30-cm (1.0-ft) ends. The uppermost soft parts were removed, divided into six equal parts, and were numbered 1 to 6 from the top. Ten single-node stem cuttings were prepared from each part, treated with the quick dip, stuck randomly in the baskets filled with a mixture of 1 Kanuma-soil (volcanic tuff with 2 to 3 mm (0.8 to 1.2 inches) diameter) : 1 peatmoss (v/v), and placed in the same frame as in 1996 but covered fully with plastic film. The propagation frame was intermittently misted, and ventilated with fans when the temperature was >35 °C (95 °F). The survival and rooting percentages, the length of the longest roots, and the number of roots per rooted cutting were recorded 60 d after the rooting treatment. All data were subjected to ANOVA, and the experimental design was a 6 (position) × 3 (time) factorial. Year (1997, 1998 and 1999) served as plots (replications) only in the experimental design. Percentage data were analyzed after being subjected to arcsin transformation.

Results and discussion

CUTTING SOURCE. In May, many root suckers sprouted and grew vigorously from the exposed part of the root (Fig. 1A). About 50 suckers over 150 cm (4.9 ft) long were produced before

November every year. If all the suckers were harvested for the single-node stem cuttings, more than 1000 cuttings could be obtained in one season, since we could prepare about 20 single-node stem cuttings per sucker. However, if all suckers produced were removed, fewer suckers would be produced the following year because additional photosynthetic supply would be unavailable.

EXPERIMENT 1. The shorter the cuttings, the better the survival and rooting percentages (Table 1, Fig. 1B). The single-node stem cuttings had more roots than the 10-cm-long cuttings (3.8 vs. 1.8, $P < 0.05$). To our knowledge, no one has reported improvements in rooting of cuttings by shortening of cutting length, although the reason why the single-node stem cuttings survived and rooted better than the other types of the cuttings was unknown. Generally, the use of single-node stem cuttings made rapid propagation from a limited number of stock plants possible (Stoutmeyer et al., 1933). The two IBA treatments had similar effects on the cuttings and failed to interact significantly with cutting length (data not shown).

Very few reports are available on the successful cutting propagation of persimmon (Machida and Fujii, 1969; Murata et al., 1983; Tukamoto et al., 1959). In all cases, etiolation of stock plants and blanching of cutting bases were necessary for good rooting. These operations require much time. Furthermore, relatively long cuttings, 10 to 15 cm (3.9 to 5.9 inches) long, were used in these studies and only a limited number of cuttings could be obtained from a tree. Moreover, many cuttings died during their acclimatization to sunlight (Tukamoto et al., 1959), and only a few cuttings survived after rooting (Murata et al., 1983). Therefore, these propagation methods have never been used commercially. In the present study, al-

Table 1. Effects of the cutting length on the survival and rooting percentages of cuttings of the persimmon rootstock (Expt. 1).

Cutting length (cm) ²	Survival (%)	Rooting (%)
3-4 (1) ^y	52	25
10 (3-4)	28	5
25 (5-7)	17	0
Linear	***	***
Quadratic	***	***

²2.54 cm = 1.0 inches.

^yNumber of buds per cutting.

***Significant at $P < 0.001$.

Table 2. Effects of the time of cutting collection and the position on sucker from which cuttings were obtained on the survival and rooting percentages of single-node stem cuttings of the persimmon rootstock (Expt. 2).

Time of cutting collection	Position of cuttings	Survival (%)	Rooting (%)	No. of roots per rooted cutting	Longest root (cm) ^z
23 June	1 ^y	97	87	3.1	11.3
	2	100	87	4.7	10.1
	3	90	80	4.3	10.8
	4	100	93	4.9	12.2
	5	93	90	6.0	12.2
	6	90	90	8.3	10.5
24 July	1	97	90	4.4	9.7
	2	93	87	3.3	9.9
	3	97	87	3.7	9.5
	4	100	100	5.0	9.5
	5	97	87	3.9	9.4
	6	100	97	4.9	10.3
20 Aug.	1	47	10	2.5	4.0
	2	33	3	2.0	8.0
	3	50	10	5.3	7.5
	4	40	13	2.7	7.7
	5	47	23	2.5	6.3
	6	53	27	3.0	4.5
Significance					
Time (T)		***	***	NS	***
Position of cuttings (P)		NS	NS	NS	NS
T × P		NS	NS	NS	NS

^z2.54 cm = 1.0 inches.

^yPosition of cuttings in suckers: 1 (uppermost part) to 6 (lowermost part).

NS,***Nonsignificant or significant at $P < 0.001$, respectively.

Percentage data were transformed to arcsin% before analysis.

though the mature fruitful tree had to be felled, the single-node stem cuttings taken from root suckers rooted well and without a cumbersome operation. Intact stock roots produced many suckers continually, and the single-node stem pieces supplied hundreds of cuttings for mass propagation. This propagation method is potentially promising for commercial use and applicable to another dwarfing rootstocks for persimmons and to rootstocks with other valuable traits, such as salt, drought and flood resistances.

Success of rooting of cuttings may be related to tissue juvenility. Juvenile tissues or cells form adventitious roots easily (Bonga, 1982). Although the rootstock used in this study was more than 33 years old, the suckers were thought to be juvenile, as Bonga (1982) suggested. Higdon and Westwood (1963) pointed out that juvenile 'Old Home' pear (*Pyrus communis* L.) cuttings, which rooted better than adult ones, were obtained from adventitious shoots forced from roots.

EXPERIMENT 2. The time of cutting collection was important for the survival and rooting of cuttings (Table 2), as has

been reported for some trees and shrubs (Howard, 1996; Murai et al., 1999; Singh et al., 1957). Several factors influencing the seasonal fluctuations of rooting ability of softwood cuttings have been suggested, such as bud dormancy (Murai et al., 1999), hardening of source shoots (Singh et al., 1957), and the rate of stockplant growth (Howard, 1996). Most of the buds on the cuttings in this study seemed to be dormant, and only a few cuttings began to produce new shoots irrespective of the time of cutting collection. The cuttings from different positions on the suckers rooted in similar percentages (Table 2). One of the important factors influencing the success of propagation of the persimmon rootstock may have been the growth rate of the suckers. Sucker growth decreased in late August when rooting ability declined.

Another important factor affecting rooting was probably the humidity in the propagation frame. Although the temperature between Expts. 1 and 2 differed little, the relative humidity in the frame was often below 45% in the daytime in midsummer in Expt. 1, whereas it was over 60% in Expt. 2. The

high humidity in Expt. 2 was maintained in the closed frame ventilated with the thermostatically controlled fans. This difference in humidity might have caused a difference in the survival and rooting percentages of single-node stem cuttings between Expts. 1 and 2. Howard and Harrison-Murray (1995) noted that high wetting in a high light intensity was necessary for rooting of the leafy cuttings of common lilac (*Syringa vulgaris* L.).

The mean of the longest roots of the cutting stuck in August was the shortest in the experiments, although the number of roots per rooted cutting was not significantly different between the time of cutting collection (Table 2). The cuttings stuck in August could not be transplanted in winter because of the poor development of their root system. In practice, considering rooting percentage and root system development, sticking cuttings late in August should be avoided.

Well-rooted cuttings that were transplanted to an outdoor nursery grew enough to be grafted in one growing season, and almost all of the grafted plants formed successful grafts (Fig. 1C).

Field performance of trees grafted on dwarfing rootstocks propagated in this way needed to be evaluated.

In conclusion, cutting propagation of the potentially dwarfing rootstock for Japanese persimmon succeeded using single-node stem cuttings taken from its root suckers. When the cuttings collected in June or July were treated with the IBA and were stuck in the propagation frame maintained in high relative humidity, more than 80% rooting was achieved. It can be speculated that the cuttings used in the present study had high rooting ability genotypically, but a subsidiary trial with another two persimmon rootstocks showed that the single-node stem cuttings collected from their root suckers also rooted well (data not shown). Hence, this clonal propagation method will make a rapid multiplication of Japanese persimmon, a difficult-to-root species, possible.

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