Factors Affecting Rooting of Japanese Persimmon Hardwood Cuttings

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Summary

The factors influencing rooting of hardwood cuttings from two cvs. of Japanese persimmon (*Diospyros kaki* Thunb.), Jiro and Nishimurawase, were studied. After the tops of micropropagated trees were removed, suckers sprouted from the roots. A quarter of the cuttings made from these basal suckers rooted. Half of the cuttings, whose basal part had been etiolated by mounding with rice husks, initiated roots. Some of the etiolated suckers which had already rooted within the mounds were easily established in a bottom-heated medium after being separated from the mother plants. Cuttings from the one-year-old shoots of micropropagated or conventionally grafted trees did not root. There was no significant difference in the rooting percentage between 'Jiro' and 'Nishimurawase' cuttings. In comparison with the control, soaking the base in 25 ppm IBA for 24 hr or dipping in 3000 ppm IBA for 5 sec did not improve the rooting percentage. Imbedding the cuttings in a rooting mixture from late-January to late-March had no effect on the rooting percentage. Almost all rooted cuttings grew well and became established. Before the rooting treatment, adventitious root primordia at various differentiation stages were found in the basal region of mounded suckers, in which a well-developed periderm and few sclerenchymatous cells were present. Root primordia were not observed in the bases of unmounded suckers or their mid-sections. The unmounded suckers had a discontinuous sclerenchymatous ring and their cuttings rooted to some degree, whereas the mid-sections of suckers had a well-developed, continuous sclerenchymatous ring and their cuttings scarcely rooted.

Key Words: *Diospyros kaki* Thunb., hardwood cutting, Japanese persimmon, mounding, root sucker.

Introduction

Japanese persimmon (*Diospyros kaki* Thunb.) is difficult to propagate by cuttings (Tao and Sugiura, 1992). Therefore, the commercial and conventional propagation is by grafting or budding onto seedling rootstocks of *D. kaki* or *D. lotus*. However, because the seedlings are genetically diverse, mature tree size is not uniform in the orchard.

Recently, various micropropagation techniques have been developed to propagate the dwarfing rootstocks and to produce own-rooted trees of commercial scion cultivars (Bellini and Giordani, 1997; Kagami et al., 1995; Matsumoto and Yamada, 1993; Tao and Sugiura, 1992). However, their application to commercial nursery stock production of Japanese persimmon cultivars has been limited because their microcuttings do not easily root (Fukui et al., 1992; Tetsumura et al., 1991).

From in vitro experiments, Tetsumura and Yukinaga (2000) found that shoots, differentiated from the root

pieces of Japanese persimmon, rooted better than those derived from shoot tips. Furthermore, we succeeded in rooting softwood cuttings of a potentially dwarfing rootstock for Japanese persimmon by using shoots which differentiated from *in vivo* roots, i. e. suckers (Tetsumura et al., 2000). In a preliminary trial, hardwood cuttings made from 'Nishimurawase' Japanese persimmon suckers initiated root, especially when their base was mounded with rice husks until early summer, similar to stool-layering; but the optimum planting time of cuttings and the best method of rooting treatment were still unclear (Tetsumura et al., 1998). In this study, we examined the effects of planting time, methods of rooting treatment, mounding, and cultivars on rooting of hardwood cuttings made from Japanese persimmon suckers, whose mother trees had been micropropagated, as compared hardwood cuttings prepared from dormant shoots of micropropagated or conventionally grafted trees.

Materials and Methods

Cutting source

In January 1991, 'Nishimurawase' Japanese persimmon trees raised by micropropagation and conventional grafting were planted in the orchard of the Experimental

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Farm of Kyoto University, as previously described (Tetsumura et al., 1999). In April 1995, the micropropagated trees, including the root shanks, were cut down just above ground level. Then, the surface soil about 1 m² around the stump and 30 cm in depth was removed, and part of the root, more than 1.5 cm in diameter, was exposed to stimulate differentiation of suckers. Micropropagated 'Jiro' trees were also planted in March 1993, and their tops were cut off in March 1997. Suckers were induced in the same way as 'Nishimurawase'. Shoots from conventionally grafted 'Jiro' trees (over 65 years) were also used for the cutting source.

Five types of shoots were prepared for the hardwood cuttings in this study. Some suckers were mounded (etiolated) with rice husks at their base from late-June; the others remained exposed (unmounded). The basal or middle part of these suckers was collected for cuttings. The cuttings from micropropagated and conventionally grafted trees were prepared from the basal part of vigorous non-bearing one-year-old shoots. All the materials were harvested in mid-December and stored at 3 °C in a refrigerator until they were treated. Cuttings with bases over 7 mm diam. were cut into 25-cm lengths and treated as described below.

Some etiolated suckers had already rooted in the mounds. After being separated from the mother plants, they were subjected to the same rooting treatments and planted in the same bottom-heated medium as in Exp. 1. Their survival (establishment) was estimated at the end of the growing season.

Effect of rooting treatments (Exp. 1)

On March 11, 1999, the bases of cuttings (10 per group) were soaked for 24 hr in 25 ppm IBA, and then for 30 min in a solution of 0.5 % benomyl. In another group, the bases were soaked in water for 24 hr and then dipped for 5 sec in a 3000 ppm IBA solution with 50% aqueous ethanol solution followed by soaking in benomyl solution, or soaked only in the benomyl solution (control). After the treatments, the cuttings were planted upright in a mixture of Kanuma soil (2 to 3 mm in diameter) and peat moss (1:1, v:v) in 20-liter plastic netted baskets 17 cm in depth, 20 cuttings per basket. The baskets with cuttings were placed under a netting coated with vaporized aluminum in a propagation frame covered with plastic film. The propagation frame was intermittently misted, and ventilated with fans, which turned on when the temperature exceeded 10 °C. Bottom heat was supplied at 28 °C by thermostatically-controlled low voltage heating wires, which were laid under the baskets. The baskets were transferred outdoors 60 days after the planting. Cuttings were checked at intervals and those which failed to root were discarded. At the end of the growing season, the number of surviving cuttings which rooted well was counted as being established; their current shoot length and number were recorded.

Effect of planting time (Exp. 2)

The experiments were conducted in 1998 and 1999 using 10 cuttings per treatment. In late-January, late-February, and late-March, the base was soaked in a 25 ppm IBA solution for 24 hr, and then in a 0.5 % benomyl solution for 30 min. Subsequently, the cuttings were planted in the same rooting medium and under the same environmental conditions as in Exp. 1. The rooting percentage, the survival rate, and the length and number of shoots were recorded at the end of growing season.

Statistical analysis

The 5 × 3 × 2 factorial with cutting source, method of rooting treatment (Exp. 1) or planting time (Exp. 2), and cultivar as main effects was analyzed by analysis of variance (ANOVA). All percentage data were subjected to arcsin transformation before being subjected to ANOVA. In Exp. 2, the years (1998 and 1999) served as the replication only in the experimental design.

Histological observations

To observe the root primordia formation and development before the rooting treatment, the basal part of the cutting derived from 'Jiro' suckers was fixed in FAA (70% ethanol: formalin: acetic acid, 90:5:5, v/v/v), treated with the softening agents according to Nakamura (1985), dehydrated in n-butyl alcohol series, embedded in paraffin, and cut transversely into 10 µm sections. The sections were stained with 0.05 % toluidine blue O and observed under a light microscope.

Results

Cutting sources

In late-April, many suckers sprouted from the exposed part of the root. Every year, each tree of both cultivars produced 20 to 30 suckers that grew well enough to be used for hardwood cuttings.

When harvested, half the etiolated 'Jiro' suckers had already rooted in the mounds; while one-third of etiolated 'Nishimurawase' suckers had formed roots. The former had 2.3 roots per sucker, and the latter had 2.4 roots. Irrespective of the IBA treatments, 80 % of the rooted suckers that were planted in the rooting medium formed new roots, and 70 % were established by the end of the growing season.

Effect of rooting treatments (Exp. 1)

Compared with the control, the IBA treatment for rooting had no effect on the rooting percentage (Table 1); cuttings of 'Jiro' and 'Nishimurawase' rooted equally well. However, the cutting source had a significant effect on the rooting. The highest rooting (52%) was achieved by cuttings made from mounded suckers. Only 3% of the cuttings from the mid-section of suckers rooted, while 27% of those from unmounded suckers

Table 1. Effects of cutting source, method of rooting treatment, and cultivar on the rooting of Japanese persimmon hardwood cuttings (Exp.1).

Factor	Rooting (%)
<i>Cutting source</i>	
Mounded suckers	52
Uumounded suckers	27
Mid - section of suckers	3
Shoots of micropropagated trees	0
Shoots of conventionally grafted trees	0
<i>Method of rooting treatment</i>	
Control	17
IBA 25 ppm for 24 hr	16
IBA 3000 ppm for 5 sec	16
<i>Cultivar</i>	
Jiro	17
Nishimurawase	16
<i>Significance</i>	
Cutting source (CS)	*** ^z
Method of rooting treatment (RT)	ns
Cultivar (CV)	ns
CS × RT	*
RT × CV	ns
CV × CS	*

^z ns, *, *** : nonsignificant or significant at $P < 0.05$ or 0.005 , respectively.

rooted. Cuttings from shoots of micropropagated trees and conventionally grafted trees did not root at all. A significant interaction was observed between the cutting source and the rooting treatment and cultivar. At the end of the growing season, almost all rooted cuttings survived; the mean number of shoots and the total length of the established cuttings were 2.4 and 41 cm, respectively. These values were not significantly affected by the cutting source, the method of rooting treatment, or the cultivar (data not shown).

Effect of planting time (Exp. 2)

The planting time had no effect on the rooting percentages (Table 2), which were similar to those in Exp. 1 for all cutting sources and cultivars. Only the cutting source showed a significant effect, and the cultivar showed a significant interaction with the cutting source. The planting time did not affect either the number of shoots or the total length of the established cuttings (data not shown). The early spring in 1998 was warmer than that in 1999, and the cuttings in 1998 sprouted 10 days earlier than those in 1999. However, there was no apparent difference in their rooting percentage (data not shown).

Table 2. Effects of cutting source, planting time, and cultivar on the rooting of Japanese persimmon hardwood cuttings (Exp.2).

Factor	Rooting (%)
<i>Cutting source</i>	
Mounded suckers	49
Unmounded suckers	24
Mid - section of suckers	2
Shoots of micropropagated trees	0
Shoots of conventionally grafted trees	0
<i>Planting time</i>	
Late - January	18
Late - February	14
Late - March	14
<i>Cultivar</i>	
Jiro	13
Nishimurawase	17
<i>Significance</i>	
Cutting source (CS)	*** ^z
Planting time (PT)	ns
Cultivar (CV)	ns
CS × PT	ns
PT × CV	ns
CV × CS	*
CS × PT × CV	ns

^z ns, *, *** : nonsignificant or significant at $P < 0.05$ or 0.005 , respectively.

Histological observations

Adventitious root primordia at various differentiation stages were visible in the basal part of cuttings derived from mounded suckers (Fig. 1A–C). These primordia originate in the phloem in association with rays beneath lenticels. The basal part of the cuttings from mounded suckers had a thick-layered periderm with well-developed phellogen and phellem and a few sclerenchymatous cells (Fig. 1D). Root primordia could not be found in cuttings from unmounded suckers. Instead, a discontinuous sclerenchymatous ring in the cortex and a several-layered, lignified periderm were present (Fig. 1E). Root primordia could not be found in the basal part of the cuttings derived from the mid-sections of suckers; they possessed a continuous, thick sclerenchymatous ring, and a well-lignified periderm (Fig. 1F).

Discussion

Although some etiolated root suckers rooted in the mounds and were easily established in the rooting medium without IBA treatment, a greater rooting percentage and root number will be needed to supply etiolated suckers as stool shoots. Apple rootstocks that have been commercially propagated by stool-layering

Fig. 1. Photomicrographs of transverse sections of basal part of hardwood cuttings derived from mounded suckers (A, B, C, D), an unmounded sucker (E), and a mid-section of sucker (F) of the Japanese persimmon 'Jiro' before the rooting treatment. (A) A root primordium in the phloem in association with xylem ray cells. (B) A well-developed root primordium under a lenticel. (C) A root primordium pushing the outer tissues. (D) A thick-layered periderm with well-developed phellogen and phellem. (E) A discontinuous sclerenchymatous ring in cortex and a several-layered and lignified periderm. (F) A continuous and thick sclerenchymatous ring and a well-lignified periderm. Abbreviations: cortex (C), lenticel (L), periderm (PE), phloem (P), ray (R), root primordium (RP), sclerenchyma (S), vascular cambium (VC), xylem (X). All scale bars = 0.1 mm.

have stool shoots with 13.4 to 58.6 roots (Doud and Carlson, 1977), and 70 to 100% rooting is considered successful (Macdonald, 1986). Girdling was reported to improve the rooting of chestnut, a difficult-to-root fruit species (Biricolti et al., 1994); it may be applied to persimmon.

Although IBA treatments did not improve rooting of persimmon hardwood cuttings (Tetsumura et al., 1998), they usually improve rooting of hardwood cuttings (Erez and Yablowitz, 1981; Howard, 1978; Howard and Nahlawi, 1969) and even with unrooted stool shoots of M.27 apple rootstock (Pontikis et al., 1979). The greatest effect of exogenous auxin on adventitious root formation occurs during early primordium development (Haissig, 1970; Norcini et al., 1985). Histological observations in this study revealed that the cuttings from mounded suckers formed adventitious root primordia before IBA treatments. These primordia probably developed into roots irrespective of IBA treatment so that exogenous auxin is unnecessary for rooting of the cuttings from mounded suckers.

However, cuttings from unmounded suckers, in which no primordium was observed, also rooted to some degree irrespective of IBA treatment. Preliminary experiments revealed that the roots of cuttings from mounded suckers were visible 20 days after planting, whereas those from unmounded suckers became visible 40 days after planting. Hence, cuttings from unmounded suckers were assumed to form root primordia after planting. A possible reason for the ineffectiveness of IBA treatment might be the degradation of IBA before it could exert an effect. The rooting of peach and apple hardwood cuttings treated with IBA occurred from 10 to 20 days after planting (Gemma et al., 1983; Howard, 1980). Further observations on root primordia formation and the concentration and metabolism of IBA in the cuttings from unmounded suckers during planting need to be examined.

The planting time of the cuttings was not an important factor for their rooting as previously observed by Tetsumura et al. (1998). The rooting ability of the hardwood cuttings of apple and plum was reported to be low in midwinter but increase in the spring (Howard, 1965; Howard, 1980; Howard and Nahlawi, 1969). Bassuk and Howard (1981) found that activities of the rooting cofactors in M.26 apple hardwood cuttings were closely correlated with the seasonal rooting response of cuttings. However, such a relationship does not seem to exist in the hardwood cuttings of Japanese persimmon.

Histological changes were educed in suckers with or without mounding. The basal part of suckers was somewhat etiolated due to shading by the foliar canopy from June to November. The basal part of cuttings made from mid-sections of suckers had well-developed sclerenchyma; those from mounded suckers had poorly-developed sclerenchyma; those from unmounded suckers had moderately-developed sclerenchyma. Many

investigators have proposed that the development of sclerenchyma reduced the rooting potential of cuttings (Biricolti et al., 1994; Doud and Carlson, 1977; Maynard and Bassuk, 1996). The development of sclerenchyma might also affect the formation of root primordia in the cuttings of Japanese persimmon after planting, because the cuttings from unmounded suckers rooted to some degree, but those from mid-sections scarcely rooted. The lenticels, paths of water and air exchange through the periderm of suckers in the mound, might help the formation of root primordia (Machida and Fujii, 1969).

In addition to the favorable effect of mounding, cuttings taken from suckers were successful for rooting, because they were physiologically juvenile (Del Tredici, 1995). Higdon and Westwood (1963) pointed out that juvenile 'Old Home' pear cuttings, which rooted better than adult cuttings, were obtained from adventitious shoots forced from roots. Softwood cuttings were propagated from a potentially dwarfing rootstock of Japanese persimmon by using the shoots derived from suckers (Tetsumura et al., 2000).

We demonstrated here that by mounding suckers, hardwood cuttings of Japanese persimmon can be successfully propagated; it is a promising propagation method. Moreover, a related trial with another Japanese persimmon cv., Hiratanenashi, hardwood cuttings were successfully propagated so that this vegetative propagation method should be applicable to other persimmon cultivars.

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カキ休眠枝挿し穂の発根に及ぼす要因について

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摘 要

カキ2品種(‘次郎’および‘西村早生’)の休眠枝挿し穂の発根に及ぼす種々の要因について調査した。地上部を切り倒した組織培養樹の根から発生したひこばえの基部を挿し穂として挿し木すると、その約4分の1が発根した。また前年のもみがらによる盛土処理により基部の黄化したひこばえを挿し穂として挿し木すると、その約半数が発根した。盛土処理したひこばえの一部は盛土中ですでに発根しており、それらは母樹から切り離した後、底熱を与えた培土中に植え付けると容易に活着した。組織培養樹や実生台接木樹の1年生枝から得た挿し穂は発根しなかった。‘次郎’と‘西村早生’との挿し穂の発根率の間に有意差は認められなかった。挿し穂基部へのIBA25 ppm 24時間浸漬処理やIBA3000 ppm 5秒間瞬間

浸漬処理は、無処理区と比較して、挿し穂の発根率を改善しなかった。1月末から3月末にかけての挿し木時期の違いは、発根率に影響を及ぼさなかった。発根した挿し穂の多くは、順調に生長し活着した。発根処理前の顕微鏡観察によって、盛土処理したひこばえから得た挿し穂の基部には、十分発達した周皮があるものの、厚壁細胞はほとんどなく、異なる分化段階の不定根原基が存在することが確認された。盛土処理しなかったひこばえやひこばえ中間部から得た挿し穂の基部に根原基は存在しなかった。前者には不連続の輪状厚壁組織が存在したが、挿し木後ある程度発根した。一方、後者には発達した輪状厚壁組織が存在し、挿し木後ほとんど発根しなかった。