Effects of Planting Time, Irrigation System, Rooting Medium, and IBA Concentration on Cutting Propagation of the Persimmon Dwarfing Rootstock ‘MKR1’

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A practical dwarfing rootstock for the persimmon (Diospyros kaki Thunb.), ‘MKR1’, is normally propagated by cuttings collected from root suckers. However, optimal conditions for propagating leaf-bud cuttings from this variety have not been researched. Thus, several methods influencing survival, rooting and root system structure were investigated in the present study. Cuttings planted in late June survived and rooted better than those planted in late July and August, and the rooted cuttings planted in late June also survived winter better. The two different irrigation methods, either a mist system, or a tray with a polyethylene tent (tray-polyethylene-tent, or TPT) did not significantly affect survival and rooting. However, the primary root length was longer in the TPT and the root dry weight was heavier under the mist system. Although the rooting medium did not significantly affect the survival of cuttings, the rooting percentage of cuttings planted in perlite was the lowest. The cuttings planted in peat in late June and placed under the mist system had adventitious root initials 17 days after planting and adventitious roots were observed on the cuttings by 22 days after planting. A quadratic regression curve predicted that the highest rooting percentage would result from treatment of ‘MKR1’ cuttings with approximately 2000 mg·L⁻¹ indole-3-butyric acid (IBA), while a different curve predicted that the lowest root number would result from treatment with approximately 1000 mg·L⁻¹ IBA. Interestingly, more than 50% of the cuttings treated with a quick dip in 0 mg·L⁻¹ IBA successfully rooted. Although the treatment with a higher IBA concentration resulted in greater root system development on the rooted cuttings, treatment with very high IBA concentrations such as 4000 or 5000 mg·L⁻¹ caused fading of leaves and dieback of cuttings.

Key Words: Diospyros kaki, indole-3-butylic acid (IBA), mist system, tray-polyethylene-tent (TPT).

Introduction

The oriental persimmon, or simply persimmon (Diospyros kaki Thunb.), originated in eastern Asia and is mainly produced in China, Japan, and Korea. The production of persimmons has recently increased worldwide, and now Azerbaijan, Brazil, Israel, Italy, Spain, and Uzbekistan each produce more than 30000 t (Food and Agriculture Organization of the United Nations, http://www.fao.org/faostat/en/#home, September 1, 2017). In the semi-arid climate of eastern Afghanistan, persimmon production has also increased, especially in Kunar Province, where there are about 100 ha of orchards growing astringent persimmon cultivars (Samadi et al., 2009). At the same time, natural wild populations of D. lotus can be found in the mountainous area of this region, and D. lotus seedlings are mainly used as rootstock for D. kaki cultivars. Recently, seedlings of D. kaki have shown potential as rootstocks for persimmon cultivars, and some non-astringent cultivars have been introduced to this region (Samadi et al., 2009). However, graft incompatibility can occur between D. lotus rootstocks and some non-astringent cultivars such as ‘Fuyu’ (Tanaka, 1930).

The persimmon is considered to be difficult to propagate using cuttings (Tao and Sugiura, 1992), and com-
mercial cultivars have usually been propagated by grafting or budding onto seedlings of _D. kaki_, _D. lotus_, _D. virginiana_, or other species. However, these seedlings are genetically diverse, which can result in different tree growth forms and non-uniform tree sizes that are unfavorable for orchard management. Recently, practical methods for persimmon propagation using cuttings have been developed (Tetsumura et al., 2000, 2001a, b, 2002, 2017), and a dwarfing rootstock, ‘MKR1’, previously known as “OD-1” or “Rootstock-b”, has been clonally propagated using leaf-bud (single-node stem) cuttings collected from root suckers (Tetsumura et al., 2003). Field evaluations of ‘Fuyu’ and ‘Hiratanenashi’ persimmon trees grafted onto ‘MKR1’ rootstocks showed that the dwarfed trees efficiently produced flowers and fruit without changes in fruit quality (Tetsumura et al., 2010, 2015). Moreover, early fruit drop, an undesirable trait for persimmon cultivation, rarely occurred in trees grafted onto ‘MKR1’ (Tetsumura et al., 2013). ‘Hiratanenashi’ trees grafted onto ‘MKR1’ showed the same photosynthetic ability as when grafted onto other rootstocks (Ishimura et al., 2015). Its developers applied to the Ministry of Agriculture, Forestry and Fisheries of Japan for variety registration of ‘MKR1’ and registration was granted in March 2015.

Although ‘MKR1’ rootstocks bring the abovementioned practical advantages to scions, optimal conditions for propagation of leaf-bud cuttings from ‘MKR1’ root suckers have not yet been investigated, except for a comparison of two types of propagation bed and two types of rooting medium (Tetsumura et al., 2003). Moreover, softwood cutting propagation of persimmon requires a mist or fog system, which demands not only special equipment, but also a stable supply of electricity and a large volume of water. Therefore, a simple system that is easy to set up should be developed for propagating softwood cuttings of persimmon in regions with poor infrastructure, such as remote areas in Afghanistan. Furthermore, a water-saving system is necessary for arid areas (Fukasawa and Ooishi, 1994). Additionally, because the irrigation system influences the water status of the rooting medium, an appropriate proper rooting medium should be selected based on the irrigation system.

Further, the optimal planting time for rooting leaf-bud cuttings of persimmon varies according to genotype. Planting in early June was the best for rooting leaf-bud cuttings of FDR-1, a rootstock of the ‘Fuyu’ persimmon tree showing a semi-dwarfing growth habit in the orchard of Fukuoka Agriculture and Forestry Research Center (Tetsumura et al., 2017), and planting in late June and July were best for rooting leaf-bud cuttings of another potentially dwarfing rootstock, “Rootstock-a” (Tetsumura et al., 2000). Planting and rooting time was also important for subsequent overwinter survival of rooted cuttings of ornamental shrubs (Hansen and Kristiansen, 2000).

An auxin, such as indole-3-butyric acid (IBA), is often applied to cuttings of difficult-to-root plants to promote rooting. ‘MKR1’ leaf-bud cuttings were treated by dipping their bases in a solution of 3000 mg·L⁻¹ IBA for 5 s. This quick-dip treatment resulted in nearly 100% rooting (Tetsumura et al., 2003). However, the optimal concentration of IBA for ‘MKR1’ leaf-bud cuttings in terms of number and length of primary roots has not previously been investigated. One study found that overwinter survival of rooted cuttings of _Stewartia pseudocamellia_ was influenced by both the quantitative and qualitative attributes of its root system, as well as by the rooting medium (Nair et al., 2008). Recently, leaf-bud cuttings of FDR-1 were found to root better when quick-dipped in 6000 mg·L⁻¹ rather than in 3000 mg·L⁻¹ IBA (Tetsumura et al., 2017). Hence, the objectives of the present study were to evaluate the influence of planting time, irrigation system, rooting medium, and IBA concentration on survival and rooting of ‘MKR1’ leaf-bud cuttings, as well as clarify the root system structure and winter survival of rooted cuttings.

**Materials and Methods**

**Exp. 1. Effects of planting time, irrigation system, and rooting medium**

‘MKR1’ nursery stocks propagated from cuttings were planted at the Field Science Center, Faculty of Agriculture, University of Miyazaki, from 2006 to 2013. The young trees were cut to just above ground level then the surface soil from approximately 0.25 m² around the stump was removed to a depth of 20 cm. Roots greater than 0.5 cm in diameter were exposed to sunlight to promote differentiation of ‘MKR1’ root suckers. Each year, all of the root suckers were severed from the roots in winter to promote sprouting of root suckers directly from the roots in spring. In late June, July, and August in 2015 and 2016, leaf-bud cuttings were collected from ‘MKR1’ root suckers. The bases of each of these cuttings were dipped in 3000 mg·L⁻¹ IBA in 50% aqueous ethanol for 5 s, and the treated and control cuttings were planted singly in 300-mL plastic pots (EG-90, 300 mL; Minamide Inc., Mie, Japan) filled with one of four types of rooting medium: perlite (φ ≤ 5 mm Nenisanso No. 1; Mitsui Mining & Smelting Co., Ltd., Tokyo, Japan), fine vermiculite (φ = 3–5 mm, TS; Asahi Kogyo Inc., Okayama, Japan), peat (pH-adjusted peat moss; Theriault & Hachey Peat Moss Ltd., New Brunswick, Canada), or Metro-Mix® 360 (Sun Gro® Horticulture Distribution Inc., Washington D.C., USA). Twelve cuttings of each medium were placed on propagation benches under intermittent mist produced by micro sprinklers (DN752A; SUN HOPE Inc., Tokyo, Japan) operating for 30 s every 15 min from 08:00–18:00 every day. The mist system was contained in a propagation house 2.7 m in height fully covered with...
polyvinyl chloride film and vaporized aluminum netting with 80% shading (Fig. 1) and ventilated with thermostatically controlled fans when the ambient air reached 38°C. The other treatments were placed into a tray-polyethylene-tent (TPT) system comprising a clear polypropylene tray (B-23; IRIS OHYAMA Inc., Miyagi, Japan) fully covered with polyethylene film 0.035 mm in thickness (Fig. 2). The bottom of the TPT was placed on a pedestal 14 cm from the ground (Fig. 1), and the TPT systems were placed in a greenhouse 3.7 m in height covered with polyvinyl chloride film under vaporized aluminum netting 1.2 m in height and ventilated with thermostatically controlled fans when the ambient air reached 25°C. The rooting medium of cuttings in the TPT was in contact with the water in the tray through drainage holes in the bottom of each plastic pot. Once a week, the TPT was opened for 30 min to replace the water. Data loggers (TR-72i; T&D Corporation, Nagano, Japan) with thermo-hygro sensors (TR-3100; T&D Corporation) measured the temperature and relative humidity in the propagation systems.

In 2015, the number of surviving cuttings, rooted cuttings, and cuttings forming callus, as well as the number and length of primary roots, were recorded two months after planting. Percentages of rooting are shown as rooted cuttings per “original” number of cuttings and per “SurvP”, the number of cuttings that survived propagation as a proportion of the original number of cuttings (Wilson and Struve, 2003). Rooted cuttings were transplanted singly into 400-mL plastic pots (EG-105; Minamide Inc.) filled with the same rooting medium as used for rooting. In fact, the rooted cuttings planted in late June were transplanted in late August, and those in late July and August were planted in late September and October, respectively. Controlled-release fertilizer (1 g/pot, Hi-control all 10; JCAM AGRI. Co., Ltd., Tokyo, Japan), containing 10% N, 10% P, 10% K, and 10% Ca, which releases for 100 days when the soil temperature reaches 25°C, was applied upon transplantation of the rooted cuttings. Pots were placed in a propagation frame covered with 50% shade netting with the plastic film open at the sides and were watered adequately. Survival of rooted cuttings over the winter was confirmed by whether the cuttings sprouted leaves in April of the following year.

In 2016, after recording the same response parameters, the roots were severed from the cuttings to measure their fresh weights. The excised roots were then dried in a constant temperature oven (DN-8; Yamato Scientific Co., Ltd., Tokyo, Japan) for 48 h at 60°C and were weighed.
Exp. 2. Histological analysis of the effects of the rooting medium

In late June 2016, several leaf-bud cuttings were collected from ‘MKR1’ root suckers, subjected to the same rooting treatments as in Exp. 1, and placed under the mist system. Cuttings planted in each rooting medium sampled at weekly intervals. After washing off the rooting medium, the basal portions of cuttings were frozen in liquid nitrogen and were then embedded in carboxymethyl cellulose gel (Super Cryoembedding Medium; Section-Lab, Hiroshima, Japan). Sections 10 μm thick were produced using a cryostat (Leica CM1850; Leica Microsystems GmbH, Wetzlar, Germany) at −20°C using Kawamoto’s film method (Kawamoto, 2003) with an adhesive film (Cryofilm Type 2C(9); Section-Lab), followed by staining with hematoxylin-eosin, and sections were observed under a light microscope (BX51; Olympus Optical Co., LTD, Tokyo, Japan).

Exp. 3. Effect of IBA concentration

In late June 2016, leaf-bud cuttings were collected from ‘MKR1’ root suckers. The bases of each cutting were dipped in 0, 1000, 2000, 3000, 4000, or 5000 mg·L$^{-1}$ IBA in 50% aqueous ethanol for 5 s, planted singly in plastic pots (EG-90) filled with peat, and then placed under the mist system. For each IBA concentration, 10 cuttings were analyzed, and the experiment was conducted twice. The number of surviving and rooted cuttings, as well as the number and length of primary roots, were recorded two months after planting, and the fresh and dry weights of roots were measured by the same methods as in Exp. 1.

Table 1. Effects of planting time, irrigation system, and rooting medium on survival, rooting, and callus formation in ‘MKR1’ leaf-bud cuttings in 2015 and 2016 (Exp. 1).

| Factor               | Survival (%) | Rooting (%) | No. of roots/rooted cuttings | Total root length (mm) | Callus (%) |
|----------------------|--------------|-------------|------------------------------|------------------------|------------|
|                      | Original     | SurvP       |                             |                        |            |
| Planting time (PT)   |              |             |                             |                        |            |
| Late June            | 94           | 76          | 80                           | 3.5                    | 416        | 68         |
| Late July            | 68           | 46          | 67                           | 3.3                    | 349        | 49         |
| Late August          | 46           | 19          | 39                           | 3.6                    | 270        | 26         |
| Irrigation system (IS) |            |             |                             |                        |            |
| Mist                 | 70           | 46          | 57                           | 3.7                    | 287        | 56         |
| TPT                  | 68           | 48          | 67                           | 3.3                    | 403        | 39         |
| Rooting medium (RM)  |              |             |                             |                        |            |
| Metro-Mix® 360       | 72           | 53          | 65                           | 3.6                    | 424        | 44         |
| Peat                 | 67           | 53          | 75                           | 4.4                    | 375        | 40         |
| Perlite              | 69           | 34          | 47                           | 2.8                    | 276        | 57         |
| Vermiculite          | 69           | 47          | 62                           | 3.1                    | 304        | 48         |
| Significance         |              |             |                             |                        |            |
| PT                   | **           | **          | **                           | NS                     | *          | **         |
| IS                   | NS           | NS          | NS                           | NS                     | **         | *          |
| RM                   | NS           | *           | **                           | *                      | NS         |            |
| PT × IS              | NS           | **          | **                           | **                     | NS         |            |
| IS × RM              | NS           | NS          | NS                           | NS                     | NS         |            |
| RM × PT              | NS           | NS          | NS                           | NS                     | NS         |            |
| PT × IS × RM         | NS           | NS          | *                            | NS                     | NS         |            |

Data were collected two months after planting from 12 cuttings per treatment in each year.

$^z$ Rooting (%) original = number of rooted cuttings as a percentage of the original number; rooting (%) SurvP = number of rooted cuttings as a percentage of those that survived cutting propagation (see Wilson and Struve, 2003).

$^y$ NS, *, and ** indicate not significant, and significant at $P<0.05$, and 0.01, by three-way ANOVA.

Statistical analysis

Data from Exp. 1 were subjected to three-way analysis of variance (ANOVA) with the following model: planting time × irrigation system × rooting medium. Replication took place over the years in the experimental design. After the data from Exp. 3 were subjected to one-way ANOVA, regression analyses were performed. All percentage data were subjected to arcsin transformation prior to performing ANOVA.

Results and Discussion

Exp. 1. Effects of planting time, irrigation system, and rooting medium

Planting time significantly affected the percentages of survival and rooting, which were the highest among the cuttings planted in late June and the lowest among those planted in late August (Table 1). The rooting percentages of leaf-bud cuttings in an earlier study of the two persimmon scion cultivars ‘Nishimurawase’ and ‘Jiro’ (Tetsumura et al., 2001b) showed the same tendency as those in the present study. In contrast, most
cuttings of a different persimmon rootstock reported by Tetsumura et al. (2010), “Rootstock-a”, could root and survive when planted in late June and July (Tetsumura et al., 2000), while FDR-1 cuttings rooted well only when planted in early June (Tetsumura et al., 2017). Tetsumura et al. (2001b) showed that the rooting ability of leaf-bud cuttings collected from root suckers could be related to the growth rate of root suckers. On the other hand, Jalil and Sharpe (1956) showed that the rooting percentage of peach cuttings was best in those planted in late June and became progressively poorer in July and August, as found for ‘MKR1’ cuttings in the present study, and indicated that potassium loss in leaves under a mist system was related to the rooting percentages. Some biochemical compounds within the cuttings could certainly affect rooting of persimmon cuttings, as was found for olive cuttings (Porfírio et al., 2016). The concentrations and responsiveness to these putative compounds could vary depending on the genotype. Alternatively, the ability of cuttings to inactivate, convert, transport, and accumulate auxins may affect the rooting percentage (Porfírio et al., 2016). Although there were no significant differences in the numbers of roots on cuttings planted at different times, roots elongated more when the cuttings were planted in late June (Table 1), possibly because the temperature at that time might have been favorable for ‘MKR1’ root growth. Roots of the cuttings planted in late June tended to be heavier at two months after planting, although there were no significant differences in fresh weights (Table 2); after transplanting in late August, the cuttings planted in late June developed roots well during their growing season, which was the longest among the three planting times. The more advanced development of the root systems of cuttings planted in late June may have contributed to their survival over winter, as cuttings planted in late June exhibited the highest percentage winter survival (Table 2). Callus formation was also better when the cuttings were planted in late June, although it should be noted that callus and root formation are independent (Hartmann et al., 2009). From these results, planting ‘MKR1’ leaf-bud cuttings in late June is the best practice for propagation.

The TPT system was an effective irrigation system for survival and rooting of ‘MKR1’ cuttings, as was the mist system (Table 1). A simple, closed chamber propagation system without mist was developed successfully for survival and rooting of the softwood cuttings of Casuarina sumatrana (Goh et al., 1995). Softwood cuttings of Clerodendron inerme in a propagation frame fully covered by polyethylene film rooted better than those in a mist system enclosed by non-woven fabric (Fukasawa and Ooishi, 1994). Although there were no significant differences, the rooting percentage of the original cuttings in the TPT was 2% lower than that under the mist system, while that of SurvP in the TPT

| Table 2. Effects of planting time, irrigation system, and rooting medium on survival over winter of rooted cuttings and fresh and dry root weights of ‘MKR1’ leaf-bud cuttings (Exp. 1). |

| Factor                  | Survival over winter (%) | Root weight (g) |
|-------------------------|--------------------------|-----------------|
|                         | per rooted cuttings | per original cuttings | Fresh | Dry |
| **Planting Time (PT)**  |                         |                  |       |
| Late June               | 87                       | 60               | 0.47  | 0.11 |
| Late July               | 28                       | 11               | 0.28  | 0.12 |
| Late August             | 30                       | 4                | 0.28  | 0.07 |
| **Irrigation system (IS)** |                         |                  |       |
| Mist                    | 49                       | 23               | 0.40  | 0.13 |
| TPT                     | 48                       | 28               | 0.29  | 0.07 |
| **Rooting medium (RM)** |                         |                  |       |
| Metro-Mix® 360          | 57                       | 33               | 0.44  | 0.12 |
| Peat                    | 48                       | 29               | 0.38  | 0.10 |
| Perlite                 | 46                       | 17               | 0.26  | 0.08 |
| Vermiculite             | 44                       | 22               | 0.30  | 0.09 |
| **Significance**        |                         |                  |       |
| PT                      | **,**                    | **               | NS    | *   |
| IS                      | NS                       | NS               | NS    | **  |
| RM                      | NS                       | NS               | NS    | NS  |
| PT × IS                 | NS                       | NS               | NS    | NS  |
| IS × RM                 | NS                       | NS               | NS    | NS  |
| RM × PT                 | NS                       | NS               | NS    | NS  |

* Data for the cuttings planted in summer 2015 were collected in April 2016. There were 12 cuttings per treatment.

* Data were collected from 1–12 rooted cuttings per treatment two months after planting in 2016.

NS, *, and ** indicate not significant, and significant at $P<0.05$, and 0.01, by three-way ANOVA.
was 10% higher. These results mean that cuttings with potential to root died in the TPT more than those under the mist system. Hence, if the negative effect of the TPT on survival of the cuttings was improved, it is likely that the rooting percentage of the original number of cuttings could also be improved. The relative humidity in the mist system on hot summer days often dropped to nearly 50% due to dry outside air that was drawn in by the cooling fans (Fig. 3). In contrast, the relative humidity in the TPT remained at over 90% even at midday, although the air temperature changes were almost the same as under the mist system. The high humidity in the TPT system may have resulted in good survival and rooting. However, TPT did not enhance rooting compared to the mist system. In an earlier study, a fog system, another closed propagation system like the TPT (Tetsumura et al., 2017), was tested for its effect on propagation efficiency. The fog system that was tested earlier improved rooting of FDR-1 cuttings (Tetsumura et al., 2017). The distinction between TPT and the fog system is that the fog system wets the leaves of cuttings and the TPT system does not. The levels of light and humidity in propagation frames are closely related to the success of survival and rooting of the cuttings (Howard and Harrison-Murray, 1995; Loach, 1977). In the present study, shading by the vaporized aluminum netting may have allowed adequate survival and rooting of ‘MKR1’ cuttings in both systems. However, survival and rooting of softwood cuttings were not affected in an earlier study when high nighttime humidity was maintained, even though the cuttings were exposed to a wide range of temperature and humidity conditions in the daytime (Fukasawa and Ooishi, 1994).

The primary roots in the TPT elongated well in the tray water through the drainage holes of the pots, while those under the mist system stopped elongation at the openings in the pots and developed secondary roots. Therefore, the structure of the root systems on cuttings rooted in the TPT differed from that of cuttings rooted under the mist system (Fig. 4), although the root number in the TPT was not significantly different from that under the mist system (Table 1). The secondary roots in the tray water did not develop well, and consequently the dry weight of roots grown in the TPT system was significantly lower than that of roots grown under the mist system. However, there were no significant differences in the fresh weight and overwinter survival of cuttings grown under the two systems (Table 2). Irrigation systems can often affect the formation of callus (Tetsumura et al., 2008), and the percentage of callus formation did differ significantly between the irrigation systems in this study (Table 1). However, as mentioned above, callus formation and rooting are not strongly correlated.

The rooting medium significantly affected all of the measured rooting parameters (Table 1), although survival was not affected (Table 2). The rooting percentages of the cuttings planted in perlite were the lowest, as indicated by the mean separation of the rooting percentages. Although there was no significant three-way interaction between planting time, irrigation system, and rooting medium, the rooting percentages of the original cuttings planted in late June in the TPT were at least 75% in all tested rooting media. In contrast, the rooting percentages of the original cuttings planted in perlite in late June and placed under the mist system were 38%. These results may indicate that more types of rooting medium are desirable in the TPT. The bottoms of the pots containing cuttings in the TPT were always immersed in water such that all types of tested rooting media, even those such as perlite which contain coarse particles, remained fully saturated due to capillary action. In contrast, the rooting media under the mist system did not retain gravitational water. Hence, under the mist system, persimmon cuttings without roots may not have been able to take up enough water.

![Fig. 3. Time course of air temperature and relative humidity under the mist systems (upper) and the TPT (lower) from July 15 to August 14, 2016 (Exp. 1).](image)

![Fig. 4. ‘MKR1’ leaf-bud cuttings planted in peat in late June and placed under the mist system (A) or in the TPT (B) for two months (Exp. 1). Bars = 10 cm.](image)
from their cut surface in the highly porous perlite and thus exhibited poor rooting, as observed for Japanese chestnut softwood cuttings (Tetsumura et al., 2008). Jalil and Sharpe (1956) found inferior rooting of the peach cuttings planted in larger-diameter perlite (φ > 3.2 mm) under the mist system. Because they found that cuttings in the smaller perlite (φ > 0.8 mm) and in the perlite mixed with peat showed higher rooting percentages, they proposed that the larger, coarser perlite held less water. The rooting medium did significantly affect the number and length of roots: the cuttings planted in Metro-Mix \textsuperscript{®} 360 or peat had more and longer roots (Table 1), whereas there were no significant differences in root weights and winter survival among media (Table 2).

On the whole, TPT was a useful and effective propagation system for ‘MKR1’ leaf-bud cuttings because the percentages of rooting and winter survival were the same as observed under the mist system. Propagation systems fully covered with polyethylene and under shading proved effective for rooting of softwood cuttings of some woody crops in other studies (Çelik et al., 1993; Goh et al., 1995; Mudge et al., 1995). TPT is simple, cost-effective, and easy to manage compared with a mist system, and could therefore be used worldwide, partly owing to its wide adaptability for use with different rooting media.

**Exp. 2. Histological analysis of the effects of rooting medium**

No root primordia were observed on the cuttings 10 days after planting, and calluses formed 17 days after planting (Fig. 5A). Adventitious root initials, easily distinguished from the callus cells, were identified at 17 days after planting in the cuttings planted in peat (Fig. 5B). In an earlier study (Tetsumura et al., 2001b), root primordia of ‘Jiro’ were visible in the vascular cambium by 20 days after planting. By 22 days after planting, adventitious roots were observed on cuttings, except for those planted in perlite, for which there were a limited number of samples and the lowest rooting percentage. In contrast, the cuttings planted in peat showed the highest rooting in Exp. 1. Therefore, cuttings treated under these conditions were used to investigate optimal IBA concentrations in Exp. 3.

**Exp. 3. Effect of IBA concentration**

Rooting percentages were significantly correlated with IBA concentration in the present study (Fig. 6). The quadratic regression curve predicted that the highest rooting percentage would be obtained when ‘MKR1’ cuttings were treated with approximately 2000 mg·L\textsuperscript{−1} IBA by quick dip. However, a different curve predicted that the lowest root number would be obtained when ‘MKR1’ cuttings were treated with approximately 1000 mg·L\textsuperscript{−1} IBA and that the number of roots would increase with increasing IBA concentration (Fig. 6). Moreover, the quadratic regression of root length on IBA concentration \[y (\text{mm}) = 0.000002x^2 - 0.0417x + 178, \quad R^2 = 0.771 \quad (P < 0.01)\] showed the same pattern as the regression of root number on IBA concentration. At the higher IBA concentrations, the rooted cuttings had more highly developed root systems (Fig. 7). The dry and fresh weights of roots increased linearly with IBA concentration \[y (\text{g, fresh weight}) = 0.0001x \quad (\text{mg·L}^{-1}) + 0.114, \quad R^2 = 0.787 \quad (P < 0.01); \quad y (\text{g, dry weight}) = 0.000004x \quad (\text{mg·L}^{-1}) + 0.0551, \quad R^2 = 0.642 \quad (P < 0.05)]. However, 2000 mg·L\textsuperscript{−1} IBA quick dip is recommended for propagation of ‘MKR1’ leaf-bud cuttings because the rooting percentage is important for propagation using cuttings. Interestingly, more than 50% of cuttings treated with 0 mg·L\textsuperscript{−1} IBA (50% aqueous ethanol quick dip) rooted. We also confirmed that 60% of ‘MKR1’ leaf-bud cuttings treated with a quick dip in distilled water rooted (data not shown). Although the persimmon has been considered difficult to root (Tao and Sugiura, 1992), ‘MKR1’ has high rooting ability, which is a preferable trait for dwarfing rootstock. However, ‘MKR1’ cuttings collected from mother stocks, or hedges, which were
cut back to a height of 40 cm each winter, were inferior in the rooting percentage and number and length of roots to the cuttings from root suckers (Tetsumura et al., 2009). Hence, the high rooting ability of ‘MKR1’ in this study was partly provided by the cutting material, or root suckers. Treatments with higher IBA concentration such as 4000 and 5000 mg·L$^{-1}$ damaged the cuttings. Some leaves faded soon after planting the cuttings, the bases of some cuttings showed dieback, and 10% of cuttings treated with 5000 mg·L$^{-1}$ died. Hartmann et al. (2009) noted that excessive concentrations of auxin can damage cuttings, and suggested optimal concentrations of IBA for use with the quick-dip method ranged from 500 to 10000 mg·L$^{-1}$. The rooting of FDR-1 persimmon cuttings was improved using 6000 mg·L$^{-1}$ IBA for the quick-dip treatment (Tetsumura et al., 2017). Therefore, the optimal IBA concentration for rooting probably varies depending on the genotype; hence, the optimal IBA concentration should be confirmed for each genotype before application.

**Conclusion**

Our study indicated that leaf-bud cuttings collected from ‘MKR1’ root suckers should be planted in late June and placed either in a TPT system or under a mist system, and that the rooting medium must be chosen depending on the irrigation system. Quickly dipping the cuttings into an IBA solution 2000 mg·L$^{-1}$ was effective for improving rooting percentages. Hereafter, TPT should be developed for large-scale clonal production of persimmons as well as other economically important tree species, especially under resource-limited conditions.

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