



Improved rooting of softwood cuttings of dwarfing rootstock for persimmon under fog irrigation



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ABSTRACT

Softwood cuttings of FDR-1, a dwarfing rootstock for persimmon (*Diospyros kaki* Thunb.), show very low rooting percentages when planted under mist irrigation. We therefore examined the possible use of a fog system in commercial softwood cutting propagation of FDR-1. Leaf-bud cuttings collected from FDR-1 hedges rooted better under the fog system, which maintained a relative humidity of approximately 100% compared to the mist system, in which humidity often dropped to less than 50% at midday. The amount of water used was lower in the fog system than in the mist system (0.18 vs. 0.89 L/h m²), thereby affecting leaf SPAD readings. Cuttings planted in early June rooted better than those planted in early July and August, and quick-dip treatment with 6000 mg L⁻¹ IBA improved rooting compared with 3000 mg L⁻¹ IBA. Cuttings collected from FDR-1 root-suckers rooted better than those from hedges, and almost all rooted cuttings, planted in June, sprouted in April of the following year. Under the fog system a practical rooting percentage of 80% was obtained when cuttings were collected from root-suckers, planted in early June, and quick-dipped in 6000 mg L⁻¹ IBA, making it a practicable irrigation system for propagation of FDR-1 leaf-bud cuttings.

1. Introduction

Persimmon (*Diospyros kaki* Thunb.) is generally considered difficult to propagate using cuttings; therefore, commercial cultivars were propagated by grafting or budding onto their seedlings, which are genetically diverse and often result in large trees (Tao and Sugiura, 1992). We recently developed an efficient softwood cutting propagation method using leaf-bud (single-node stem) cuttings, which revealed successful rooting of some cultivars and potentially dwarfing rootstocks under a mist irrigation system (Tetsumura et al., 2000, 2001, 2002, 2003). This vegetative propagation method allowed propagation of potentially dwarfing rootstocks and made it possible to compare trees grafted on clonal rootstocks in the field.

Field evaluations of trees grafted onto 'MKR1' rootstock (previous name "Rootstock-b") propagated from cuttings revealed efficient flower and fruit production on the dwarfed trees (Tetsumura et al., 2010, 2015a), and 'MKR1' cuttings have a high rooting ability (Tetsumura et al., 2003), which is a preferable trait for dwarfing rootstock. Meanwhile, apple cultivation using dwarfing rootstocks is expanding worldwide thanks to the development of numerous dwarfing rootstocks. Consequently, an adaptability test of dwarfing rootstocks for persimmons, including 'MKR1' was initiated in 17 public research

organizations in Japan in 2016. However, cuttings from some dwarfing rootstocks show low rooting percentages, and improvements in vegetative propagation are therefore needed for expansion of dwarf persimmon tree culture.

'Fuyu' persimmon trees grafted onto FDR-1 (Fukuoka Dwarfing Rootstock No. 1) in the orchard of Fukuoka Agriculture and Forestry Research Center, Japan, showed a semi-dwarfing growth habit. After cutting from the rootstock, the roots differentiated root-suckers. Explants (buds) from these root-suckers were subsequently collected and micropropagated. Young 'Taishu' persimmon trees grafted onto the micropropagated FDR-1 rootstocks showed dwarfed growth (Haranoushiro, personal communications). We therefore attempted softwood cutting propagation of FDR-1 using a mist system; however, the rooting percentages of the leaf-bud cuttings were very low (10–20%). Softwood cuttings of Japanese chestnut (*Castanea crenata* Sieb. & Zucc.) irrigated with a micromist system in a closed chamber, in which the relative humidity was higher than that under the mist system (> 60% vs. < 50% at midday in summer), showed improved rooting (Tetsumura et al., 2008), and in a preliminary experiment, rooting percentages of FDR-1 cuttings irrigated with the same micromist system were slightly increased. These findings suggest that the relatively higher humidity improved rooting of the FDR-1 cuttings and Japanese

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chestnut cuttings.

Fog systems produce relative humidity conditions close to 100% (Hartmann et al., 2009). The difference between fog and micromist is the average droplet diameter (15 vs. 40 μm) (Mee, 1994), while mist droplets are generally 50–100 μm . Droplets smaller than 40 μm stay airborne as fog, while those larger than 40 μm tend to settle and condense as water on the leaf surface (Hartmann et al., 2009). Although *Prunus* and *Castanea* rootstocks, which are thought to be difficult-to-root, have been successfully propagated using a fog system (Štefančíč et al., 2005, 2006, 2007), few reports have compared rooting of cuttings in fog systems versus those in mist systems. Leafy cuttings planted in fog systems do not always root better than those in mist systems (Harrison-Murray et al., 1988). Moreover, these systems also affect better survival after rooting as well as more successful overwintering (Cameron et al., 2001). Various types of fogging equipment have recently been manufactured for use in greenhouse cooling, some of which are simple and compact, designed for small spaces. We developed a fog system aimed at improved rooting of cuttings deemed difficult to root and examined its application using FDR-1 cuttings, which did not root well under the mist system.

FDR-1 was micropropagated as described above, one of the obstacles of which is the low rooting ability of micropropagules. The optimal concentration of indole-3-butyric acid (IBA) required for root induction via quick-dip treatment was therefore investigated. FDR-1 microcuttings treated with 500 mg L^{-1} IBA for 5 s rooted better than those treated with 250 and 1000 mg L^{-1} (Tetsumura et al., 2015b), although Tao and Sugiura (1992) suggested that 250 mg L^{-1} IBA was the optimal concentration for rooting of persimmon microcuttings.

In terms of practicality, at least 75% of cuttings should be available as nursery stocks one year after planting in rooting media. A high rooting percentage and low mortality after rooting are therefore necessary prerequisites. We therefore investigated the possibility of commercial softwood cutting propagation of FDR-1 using the developed fog system combined with the other improvement factors: planting time, IBA concentration and cutting source.

2. Materials and methods

2.1. Plant materials

FDR-1 scions derived from micropropagated trees in the Fukuoka Agriculture and Forestry Research Center were grafted onto three-year-old *D. kaki* seedlings planted in the Field Science Center, Faculty of Agriculture, University of Miyazaki, in April 2006. They were cut back to a height of 40 cm each winter for establishment of hedges to provide mother stocks for cuttings. A micropropagated FDR-1 nursery stock was also planted next to the hedges in December 2008. In March 2011, the young tree was cut to just above ground level and then the surface soil of approximately 0.25 m^2 around the stump was removed to a depth of 20 cm. Roots > 0.5 cm in diameter were exposed to sunlight to promote differentiation of FDR-1 root-suckers, which had supplied sufficient cuttings for experiments since 2014.

2.2. Experiment of cuttings from hedges (Exp. 1)

Leaf-bud cuttings 3–4 cm in length were obtained from the FDR-1 hedges in early June, July, and August from 2011 to 2014. Every year, 360 cuttings were used. They were dipped in 50% aqueous ethanol with 3000 or 6000 mg L^{-1} IBA for 5 s, planted singly in a 300-ml plastic pot (EG-90, Minamide Inc., Mie, Japan) filled with Metro-Mix[®] 360 (Sun Gro, Horticulture Distribution Inc., Washington D.C.) as bed soil then placed under vaporized aluminum netting with 80% shading in a greenhouse. Under the mist system, the greenhouse was intermittently misted (30 s misting followed by 15 min pause) from 08:00–18:00 using micro sprinklers (DN752A, SUN HOPE Inc., Tokyo, Japan). Ventilation was provided by fans located on the upper sides of the greenhouse when

ambient air reached 38 °C. Under the fog system, intermittent fogging (30 s fogging and 1 min pause) was carried out between 07:00–18:00 using humidification spray nozzles (Mini Fogger II, Spraying Systems Co., Illinois), which produce very fine water droplets 10 μm in diameter, in a closed chamber covered with plastic film. No fans were used in the fog system, but the top windows of the greenhouse sheltering the chamber were opened when the ambient air reached 30 °C.

In preliminary experiments, relative humidity in the fog system decreased at very hot midday temperatures in summer. A temperature sensor was therefore installed to turn on continuous fog spraying when the upper side of the chamber was over 40 °C. Data loggers (TR-72i, T & D Corporation, Nagano, Japan) with thermo-hygro sensors (TR-3100, T & D Corporation) were used to measure the temperature and relative humidity in both systems at five-minute intervals. In the mist system, the sensor was placed just above the micro sprinklers to prevent disturbance by the droplets, while in the fog system they were placed next to the cuttings. Ten cuttings per treatment were planted each month (June, July, and August) in each year. The number of surviving cuttings, sprouting cuttings, rooted cuttings, and cuttings forming a callus as well as the number and length of roots were recorded two months after planting. The percentages of rooting were shown as per “original” number of cuttings and per “SurvP”, the number of cuttings surviving the propagation phase as a proportion of the original number (Wilson and Struve, 2003). Rooted cuttings were transplanted singly in 400-ml plastic pots (EG-105, Minamide Inc., Mie, Japan) filled with Metro-Mix[®] 360. 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 was released for 100 days when the soil temperature reached 25 °C. Pots were placed under a black shade netting in a propagation frame covered with plastic film that open at the sides, which provided them with 50% irradiance, and were watered by sprinklers every day. Survival of rooted cuttings was confirmed by sprouting in April of the following year.

2.3. Experiment of cuttings from hedges and root-suckers (Exp. 2)

Leaf-bud cuttings were prepared from hedges and root-suckers in early June 2014, 2015, and 2016. Ten cuttings per treatment were treated as above with mist and fog irrigation and two IBA concentrations. Soon after transplanting to 400-ml pots, leaf SPAD readings of three spots per leaf were taken using a chlorophyll meter (SPAD-502, Minolta Camera Co., Tokyo, Japan). Survival of rooted cuttings was investigated in cuttings planted in 2014 and 2015.

2.4. Statistical analysis

Data were subjected to three way analysis of variance (ANOVA) using planting time (Exp. 1) or cutting source (Exp. 2) \times irrigation system \times concentration of IBA. Years (4 in Exp. 1, and 3 in Exp. 2) served as the only replication in the experimental design. All percentage data were subjected to arcsin transformation before ANOVA. The three means (planting time) were separated using Fisher's least significant difference (FLSD) test at 5% significance.

3. Results

Cuttings grown under the fog system were surrounded by fog even in the presence of midday heat, allowing relative humidity of approximately 100% to be maintained throughout (Fig. 1). On the other hand, the relative humidity in the mist system often dropped to less than 50% on hot sunny days, the dry outside air being drawn in by the cooling fans. Moreover, in the hot midday sun, temperatures often reached more than 40 °C. In contrast, under the fog system, although no fans were used in the chamber, temperatures did not exceed 40 °C, probably because of the cooling effect of the fog as a result of vaporization. In addition, temperature and relative humidity fluctuated

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