Hardwood and softwood cutting propagation of MKR1, persimmon dwarfing rootstock©

Z. Hejazi, S. Ishimura, C. Honsho and T. Tetsumura[a]

Faculty of Agriculture, University of Miyazaki, 1-1 Gakuen Kibanadai-Nishi, Miyazaki 889-2192, Japan.

INTRODUCTION

'MKR1' is a newly released rootstock for persimmon (*Diospyros kaki* Thunb.). In spite of dwarfism, some cultivars grafted onto it showed early fruit bearing and less physiological fruit drop (Ishimura et al., 2013). Cuttings of the persimmon were speculated to be difficult-to-root (Tao and Sugiura, 1992). However, recent studies indicated their high rooting percentages, particularly with cuttings from root-suckers (Tetsumura et al., 2000, 2001a, b, 2003). In the present studies, we showed some effective rooting treatments for the 'MKR1' adventitious root formation.

MATERIALS AND METHODS

Experiment 1: hardwood cutting

In late March 2015, 'MKR1' less than 10 years-old trees raised by micropropagation and seedlings, planted in the field science center of University of Miyazaki (32.0167°N, 131.3500°E, 100 m a.s.l., 3100 mm average annual rainfall) were cut back to the ground level. A shallow pit about 10 cm in depth and 30 cm^2 around the stump was dug. The roots were bared to stimulate sucker differentiation in the presence of sunlight (Figure 1A).

In mid-June 2015, some suckers were mounded (etiolated) (Figure 1C) with rice husks and the rest remained unmounded (exposed) (Figure 1B). Rice husks can be easily eroded with heavy wind or rain, hence, they were covered by green plastic netting around the root-suckers. It is woven very open, allowed free penetration of sun, air, and rain drops to the mounded substrate. During all this period, no irrigation was conducted in order to wet the mounded medium, because adequate rainfall was taking place throughout all the growing season (Figure 1).

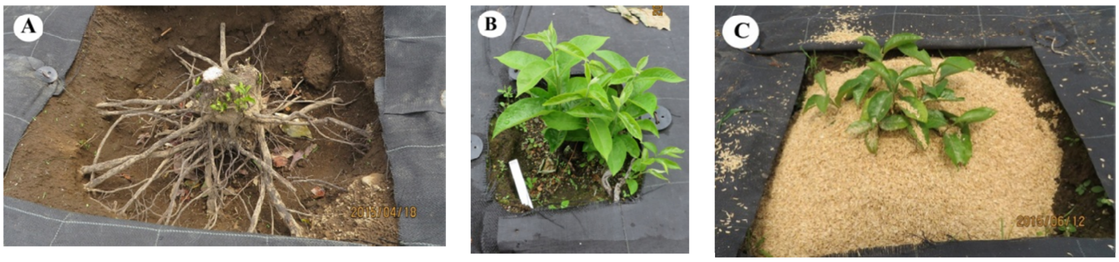

Figure 1. Experiment 1: Sucker stimulation from 'MKR1' roots: (A) A shallow pit around the stump; (B) unmounded suckers; (C) mounded suckers.

In late January 2016, rice husk was gently removed from the dormant root-suckers. None of the mounded and unmounded root-suckers had developed roots before separating from the mother plants. The suckers were de-attached, labeled, and then measured for diameter and length. The cuttings varied in terms of thickness and length, where longer shoots were clipped at 30 cm. Before being set into the rooting medium, the cuttings were re-cut straight at the proximal end to a length of 2-4 mm and angled to the distal end. The 'MKR1' cuttings were collected from mounded and unmounded (control), and 'Jiro' from mounded and hedges (control). Half of the mounded 'MKR1' suckers were wounded with pruning shears on two opposite sides of the stem by longitudinal incisions about 1-2 cm in

[a]E-mail: tetsumur@cc.miyazaki-u.ac.jp

length and 3-5 mm in width. The basal wound along with intact bark of each cutting was treated with 0.5% IBA in talc after once briefly immersed in the tap water. The prepared cuttings were planted vertically to a depth of 5 cm into bottom heated plastic net baskets (inside = 12.8 cm wide × 41 cm long × 7 cm deep), filled either with a moist mixture of peat and bora-tsuchi (1:1, v:v) or peat alone. The inserted cuttings spaced uniformly in rows ≈5 cm from the sides of the basket and being apart from each other. The basal heating mat was set to warm the substrates to 28°C. The cuttings were placed under netting, coated with vaporized aluminum in a propagating frame covered with plastic film. The propagation house was ventilated with fans when the ambient temperature rose above 20°C. The mean temperature in the substrate was 22±2°C. The mean air temperature in the propagation house was 25°C during daylight hours. Air temperature was recorded every 20 min for the duration of the experiment using a data logger (TR-72i, T&D Corporation, Japan). The substrate was watered when needed.

The experiment layout was 2×5 factorial, with 12 cuttings per each treatment. Data on percentage rooting, root number, leaf area, leaf number, callus, root and shoot lengths, fresh weights, and dry weights were recorded after 4 months from planting (late May). Cutting scored as “rooted” when an adventitious root had emerged from the stem. The roots were cut off from the stem, washed and then fresh weights were measured. Root and shoot dry weights were determined by weighing the dry mass after oven drying at 60°C for 48 h. Leaf area was calculated with the help of ImageJ application software (Rasband, 1997-2012) after scanning with a photocopier machine (Satera MF8570Cdw, Canon Inc. Japan).

Data were subjected to analysis of variance (ANOVA) using the general linear models procedure using SPSS (16.0). Mean separation was performed using Tukey’s honestly significant difference (HSD) method at $p \leq 0.05$ or 0.01.

Experiment 2: softwood cutting

In late June, late July and late August of 2015, single-node stem cuttings with one leaf and one bud (Tetsumura et al., 2000) were collected from ‘MKR1’ actively growing root-suckers. After excising, the basal part of the each cutting was immediately dipped for 5 s in the prepared 3000 ppm indole-3-butyric acid (IBA) 50% ethanol aqueous solution. The cuttings were planted singly in the plastic pots (EG-90, 300 ml, MINAMIDE Inc., Japan), filled with pre-soaked perlite, vermiculite, peat, or Metro-Mix®360 (Sun Gro, Horticulture Distribution Inc., Washington DC). Twelve replications of each medium pot were placed under intermittent mist, operated 30 s every 15 min during daytime and ventilated with thermostatically controlled fans when the ambient air reached 38°C. The other replications were put in the flood-tray polyethylene tent (FTP), which was fully covered with transparent plastic film but opened for 30 min ventilation once a week. The lowest part of the pots in FTP was always in the tray water, as substrate absorbed water by capillary action. Both systems were placed under netting, coated with vaporized aluminum in the propagating frames covered with plastic film. The data loggers measured the temperature and relative humidity in the systems.

Rooted cuttings were carefully washed in a bucket having tap water to remove substrate as much as possible. Data of root number, root length, survival, and callus for each experiment were assessed 60 days after planting. Cuttings were scored rooted, having length ≥1 cm with or without leaf retention. Those that were severely rotted or blackened were defined as “dead.”

The layout for the experiment was a 3 (planting time) × 2 (irrigation systems) × 4 (rooting medium) factorial. The method of data analysis was the same as Experiment 1.

After root assessment, rooted cuttings of the each propagation substrate were re-potted (EG-105, 400 ml, MINAMIDE Inc. Japan) into the same substrate as they were for root formation, and placed under direct misting for 2 days. Thereafter, cuttings were weaned from the mist bench, (10N-10P-10K) controlled-release fertilizer (Hi-control all 10, JCAM AGRI. Co., Ltd., Japan) was added at 1 g pot^{-1}, and remained in the ambient greenhouse for 11 more days. After passing these days, rooted cuttings then were transferred to the adjacent minimally shaded (50%) greenhouse which was misted once in the midday. Unless otherwise mentioned, mist was conducting more in accordance with prevailing irradiance and temperature conditions in a given day.

The experiments lasted in the minimally shaded greenhouse until winter passed and acclimatization survival was checked in April of the following year. The hardening process for the rest of two other months was conducted in the same manner, however, in order to encourage natural dormancy, amount of the fertilizer gradually reduced to 0.5 and 0.25 g to the late July and August planting.

Experiment 3: different IBA concentrations affecting adventitious roots of the 'MKR1' softwood cuttings

In late June of 2016, single-node stem cuttings with one leaf and one bud were collected from 'MKR1' root-suckers. Five IBA concentrations (1000, 2000, 3000, 4000, and 5000 ppm) and a solvent control were utilized. For each concentration, IBA was dissolved into a 100% ethanol solution and then diluted with the same volume of distilled water. The IBA was applied for 5 s to the basal portions of the each cutting. Unless otherwise mentioned, cuttings for the control treatment were dipped only into a 50% ethanol-distilled water solution. The cuttings were inserted singly into the plastic pots (EG-90), filled with a presoaked peat, and then placed under vaporized aluminum netting in a propagation frame covered with plastic film. The propagation frame was intermittently misted (30 s mist every 15 min in the daytime) and was ventilated with fans when the ambient air reached 38°C.

A randomized completely block design (RCBD) with two replications and 10 cuttings per each replication were used. The rooting percentage, survival, callus, root number, root length, root fresh and dry weights were measured 2 months after planting. A cutting was considered rooted if a minimum of one root ≥1 cm in length was present.

The results were statistically analyzed using SPSS 16.0. Percentage data were arcsine transformed before conducting ANOVA. Means separations were carried out by Tukey's HSD. Regression for the selected variables was performed combining data for each parameter collected from two replications.

RESULTS

Experiment 1

Rooting and callus percentages of 'MKR1' mounded and control (Figure 2) were compared with 'Jiro' mounded and control (Table 1). Later, a one way ANOVA was conducted separately for 'MKR1' mounded, control and wounding preceded IBA treatments (Table 2). Although 'Jiro' and 'MKR1' received the same treatments in terms of cuttings source and substrates only wounding and IBA were applied to the 'MKR1' cuttings. The data for the shoot parameters along with leaf number and leaf area encompassed both rooted and un-rooted cuttings are shown in Table 2.

There were significant differences in the cultivar, cutting source and substrate, and the interactions with respect to rooting percentage. Callus was not affected by any of the factors with the exception of the interaction between the cultivar and cutting source (Table 1). The cuttings treated with mounding rooted significantly better than the controls in both cultivars. The highest rooting (92%) and callus (67%) percentages occurred in the mounded 'Jiro' planted in the mixture (Table 1). 'MKR1' also formed maximum callus (33%) in the mixture, but the rooting percentage was as high as that in peat induced in the mounded cuttings (17%). Despite that peat medium was less effective with respect to rooting and callus, it had very sticky particles which didn't allow deep roots to pull or separate easily during harvesting.

Figure 2. Experiment 1: Hardwood cuttings of the 'MKR1' root-suckers, inserted into mixture substrate and measured four months after planted in January 22, 2016: (A) Unmounded treatment; (B) Mounded treatment; (C) Mounded received wounding plus 0.5% IBA in talc. Ruler = 30 cm.

Table 1. Effects of cultivar, cutting source and substrate on rooting and callusing of persimmon hardwood cuttings. The data were collected four months after planting.

Cultivar	**Cutting source**	**Substrate**	**Rooting (%)**	**Callus (%)**
Jiro	Control	Peat	8	8
		Mixture	0	25
	Mounded	Peat	25	33
		Mixture	92	67
MKR1	Control	Peat	0	25
		Mixture	8	33
	Mounded	Peat	17	8
		Mixture	17	8
Significance				
Cultivar (Cv)			**[1]	ns
Cutting source (Cs)			**	ns
Substrate (S)			**	ns
Cv x Cs			**	**
Cs x S			**	ns
S x Cv			*	ns

[1]ns, **, *: nonsignificant or significant at $p \leq 0.01$ or 0.05, respectively.

The highest callus formation was observed in the highest percent rooting, but the callus developed well even when no roots or fewer roots formed (Table 1). Mounding dramatically enhanced 'Jiro' rooting and callusing, but 'MKR1' appeared to be still difficult-to-root.

The addition of wounding practice with IBA 0.5% in talc to the mounded root-suckers significantly increased 'MKR1' rooting percentage, callus percentage, leaf number, leaf area, shoot fresh and dry weights over the control and the mounding alone (Table 2). The root length, shoot length, root fresh and dry weights failed to increase with wounding, since those were not significantly affected by the treatments. Although there was no significant difference between the substrates, the mixture medium tended to promote root and shoot development (Table 2).

Table 2. Effects of cutting source and substrate on root and shoot parameters of 'MKR1' hardwood root-sucker cuttings.

Treatment	Rooting (%)	Callus (%)	Root no.	Root length (mm)	Root Fwt (g)	Root Dwt (g)	Shoot length (mm)	Shoot Fwt (g)	Shoot Dwt (g)	Leaf no.	Leaf area (cm^2)
Cutting source											
Control	4 b[1]	8 b	3 a	83 a	0.04 a	0.02 a	59.1 a	0.75 b	0.27ab	5.38 b	5.99 b
Mounded	17 ab	29 b	2 a	218 a	2.46 a	0.41 a	52.6 a	0.69 b	0.24 b	4.78 b	4.78 b
M + W + IBA[2]	42 a	79 a	3 a	303 a	3.34 a	0.52 a	78.4 a	1.57 a	0.49 a	10.57a	10.57a
Substrate											
Mixture[3]	28 a	36 a	3 a	299 a	3.63 a	0.58 a	70.7 a	1.17 a	0.39 a	7.05 a	7.14 a
Peat	14 a	36 a	3 a	199 a	1.62 a	0.25 a	56.0 a	0.83 a	0.27 a	7.02 a	7.29 a

[1]Mean value within each factors in the same column, followed by the same alphabet are not significantly different at $p \leq 0.05$ level (HSD).

[2]M = Mounding, W = Wounding, IBA = Indole-3-butyric acid.

[3]Mixture (Peat: Bora-tsuchi; 1:1).

Experiment 2

The cutting collection time had significant influence over on all parameters except of root number, which was affected by none of the factors (Table 3). The highest rooting percentage occurred in late June (70%), followed by late July (34%) and late August (17%). A significant increase regarding survival, callus and root length was also evident to the June planting. There was no significant difference between mist and FTP with the exception of callus and root length (Table 3). Similarly, substrate affected percentage rooting, callus, and root length at $p \leq 0.05$, however, it didn't show a significant difference for the survival and root number. The highest rooting percentage was obtained by peat (50%) and the lowest was observed in perlite (28%). Conversely, callus was significantly higher in perlite (51%), but it was significantly poor in the peat (25%). Perlite also returned significantly lower root length, whereas Metro-Mix® produced the maximum.

Table 3. Effect of planting time, irrigation system, and substrate on rooting of 'MKR1' softwood cutting. The data were collected 60 days after planting for each experiment.

Factor	Rooting (%)	Survival (%)	Callus (%)	Root no.	Root length (cm)
Planting time					
Late June	70 a[1]	93 a	65 a	3.9 a	466.3 a
Late July	34 b	61 b	42 b	3.1 a	265.5 b
Late August	17 c	63 b	9 c	4.0 a	230.2 b
Irrigation system					
Mist	39 a	71 a	48 a	3.7 a	297.4 b
FTP	42 a	74 a	29 b	3.7 a	450.6 a
Substrate					
Metro-Mix®	40 ab	75 a	32 bc	4.0 a	516.1 a
Peat	50 a	71 a	25 c	4.2 a	338.3 ab
Perlite	28 b	71 a	51 a	2.9 a	287.0 b
Vermiculite	43 ab	72 a	46 ab	3.4 a	348.5 ab

[1]Mean value within each factors in the same column, followed by the same alphabet are not significantly different at $p \leq 0.05$ level (HSD).

Irrigation systems and substrate had pronounced effects on June rooting at $p \leq 0.01$ (Table 4) (Figure 3). The mean percent rooting of the FTP was significantly greater than that of the mist. The highest rooting percentage was achieved in peat (100%) and the lowest was also yielded under mist in the perlite (17%). In spite of vermiculite which stimulated 92% rooting, other propagation substrates either displayed the maximum rooting (≥75%) under the FTP system.

Although none of the factors affected survival per original cutting (SurvP), there was a significant difference in survival per rooted cutting (SurvW) between the substrates (Table 4). One hundred percent acclimatization survivals of rooted cuttings was observed in perlite and Metro-Mix® substrates in both systems.

Average day/night temperatures in the mist house and FTP were 23/25, 27/28, and 29/31°C for June, July, and August, respectively. Likewise, average day/night relative humidity in the mist house and FTP were 97/99, 93/99, and 89/96% for June, July, and August, respectively.

Table 4. Effect of irrigation system and substrate on June rooting and survivals of 'MKR1' softwood cutting.

Irrigation system	Substrate	Rooting (%)	Survival[2] (%)	
			SurvP	SurvW
Mist	Perlite	17	75	100
	Vermiculite	42	83	60
	Peat	100	100	75
	Metro-Mix®	75	100	100
FTP	Perlite	75	100	100
	Vermiculite	92	100	82
	Peat	75	100	78
	Metro-Mix®	83	83	100
Significance				
Irrigation system		**[1]	ns	ns
Substrate		**	ns	*

[1]ns, **, *: nonsignificant or significant at $p \leq 0.01$ or 0.05, respectively.

[2]Survival (%) SurvP = the number of cuttings surviving the propagation phase as a proportion of the original number; Survival (%) SurvW = the number of survival as a proportion to the rooted cuttings. See Wilson and Struve (2003) and Tetsumura and Yamashita (2004).

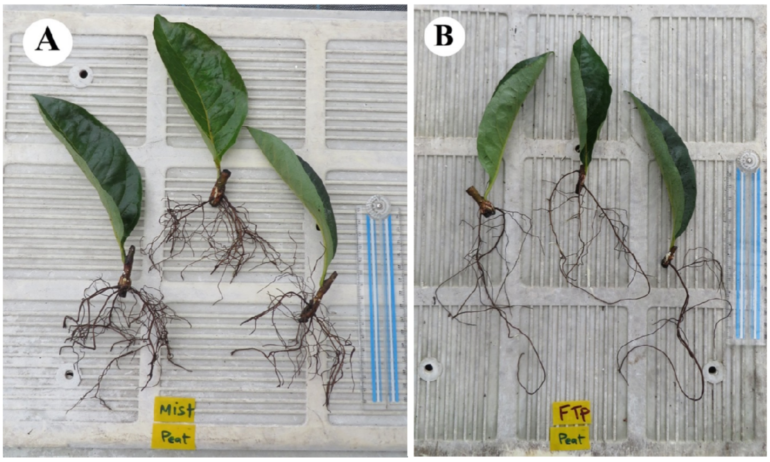

Figure 3. Experiment 2: Softwood single-node cuttings of the 'MKR1' root-suckers: (A) Roots formed under intermittent mist; (B) Roots under FTP condition. Ruler = 15 cm.

Experiment 3

The rooting percentage, root number, root length, root fresh and dry weights were significantly different at $p \leq 0.05$ (Table 5). Although callus and survival were not affected by any of the treatments, it tended to decrease gradually in high IBA concentrations. The greatest percentage rooting (90%) occurred in 2000 ppm and the lowest (30%) observed in 5000 ppm IBA. On the contrary, root number and root length continued to increase throughout the range of IBA concentration from the control to a remarkable increased in 5000 ppm (Table 5) (Figure 4). In addition, root fresh and dry weights were also significantly higher in 5000 ppm than did those in the control (Table 5).

There were significant quadratic relationships at $p \leq 0.05$, between rooting percentage (y) and IBA concentrations (x), root number (y) and IBA concentrations (x), and rooting percentage (y) and root number (x). Equations and R^2 values for the above correlations were [$y = -0.000006x^2 + 0.023x + 59.29$, $R^2 = 0.76$], [$y = 0.0000004x^2 - 0.0009x + 2.53$, $R^2 = 0.75$], and [$y = -0.803x^2 + 2.75x + 67.89$, $R^2 = 0.40$], respectively.

Table 5. Effect of different IBA concentrations on survival, callus and rooting traits of the 'MKR1' softwood cuttings. The data were collected 60 days after planting.

Treatments	Survival (%)	Callus (%)	Rooting (%)	Root no.	Root length (mm)	Root Fwt (g)	Root Dwt (g)
IBA (0 ppm)	100 a[1]	100 a	55 abc	2.3 b	144.6 b	0.10 b	0.033 b
IBA (1000 ppm)	100 a	100 a	80 ab	2.3 b	221.3 b	0.23 b	0.126 ab
IBA (2000 ppm)	100 a	100 a	90 a	2.4 b	224.2 b	0.45 ab	0.160 a
IBA (3000 ppm)	95 a	90 a	70 abc	3.6 ab	277.9 b	0.44 ab	0.154 a
IBA (4000 ppm)	95 a	75 a	45 bc	5.6 ab	412.2 ab	0.42 ab	0.137 ab
IBA (5000 ppm)	90 a	75 a	30 c	7.7 a	605.0 a	0.77 a	0.237 a

[1]Mean value within each factors in the same column, followed by the same alphabet are not significantly different at $p \leq 0.05$ level (HSD).

Figure 4. Experiment 3: Depicts treatment differences about number of roots per cutting: (A) Cuttings treated with 2000 ppm; (B) Cuttings with 5000 ppm. Ruler = 15 cm.

DISCUSSION

Experiment 1

Our main purpose of this study was to optimize 'MKR1' rooting. In general, the whole result of the 'Jiro' was a kind of control, used to know precisely about 'MKR1' rooting difficulties.

The rooting responses between 'Jiro' and 'MKR1' were statistically distinct, but both were considerably improved by mounding. Tetsumura et al. (2001a) demonstrated 52% rooting for the mounded suckers of 'Jiro' and 'Nishimurawase', which was significantly higher than that of control (27%). Likewise, the mounded micropropagated stock plant of 'Nishimurawase' rooted (47%), as high as the mounded root-suckers did, while the unmounded micropropagated stock plants and the unmounded root-suckers were respectively no and less responsive to the rooting (Tetsumura et al., 2002). As for the useful dwarfing rootstock for 'Fuyu' Japanese persimmon, *Diospyros rhombifolia* Hemsl., hardwood cuttings from basally etiolated root-suckers rooted better than those of the basal or middle suckers (Tetsumura, 2000). In a number of woody plant species, etiolation as a pretreatment has been beneficial to improve rooting. Anatomical changes associated with etiolation are delayed lignification of pericyclic cells (Amissah et al., 2008). Numerous studies have proposed a correlation between difficulty in rooting and the presence of a pericyclic sclerenchyma layer. A continuous sclerenchyma layer might act as a physiological barrier to the adventitious root initiation or as a mechanical barrier to root emergence (Amissah et al., 2008). Tetsumura et al. (2001a) showed that a moderately developed sclerenchyma of the unmounded 'Jiro' sucker was connected with low rooting, but a poor developed sclerenchyma of the mounded cutting was associated with significantly higher rooting. Thus, no or scarce rooting of the unmounded suckers or hedges in our study would be related to a wide range of lignification.

It is well known that the substrate has influence over adventitious root formation. In our study, cuttings in two-component mixture of peat moss and bora-tsuchi rooted and callused better than in pure peat. Generally, peat moss was noted for low callus and root rates in proportion to the mixture substrate. Hechmi et al. (2013) reported higher percentage rooting for the olive semi-hardwood cuttings in two component media of perlite and sand, whereas peat moss returned poor rooting. Similarly, Ercisli et al. (2002) investigated rooting characteristics of *Actinidia deliciosa* 'Hayward' hardwood cuttings in perlite, peat, sawdust, peat + sawdust (1:1), and peat + perlite (1:1). The highest rooting percentage was found in two component medium of peat + perlite and peat + sawdust. Hardwood cutting of figs (*Ficus carica* L. 'Roxo de Valinhos') also indicated best result in the soil/sand (v:v, 1:1) for the root and shoot parameters (Antunes et al., 2003). The author added that soil alone decreased root and shoot development. In order to improve aeration, nutrient uptake and water retention of the propagation medium, investigators give preferences to the mixture instead of using a single substrate (Aklibasinda et al., 2011; Al-Salem and Karam, 2001; Al-Saqri and Alderson, 1996).

The physical properties of the different propagation substrates (water content, aeration capacity and drainage) together or separately affected adventitious root and shoot development in several studies. Yingqiang et al. (2007) assessed sand, perlite and peat mediums and mixture of them to the grapevine seedless cultivars hardwood cuttings. He observed high percentage rooting, quantities and strong adventitious roots to the cuttings planted in peat: sand: perlite (volume as 1:1:1) mixture. Sand may be linked to excessive aeration and low capacity of water retention, however, peat serves vice versa. Hechmi et al. (2013) found peat moss unsuitable because it produced large swellings at the bases of the cutting and occasional apical necrosis. Though, we did not observe such a symptom for 'MKR1' or 'Jiro', but soft particles of the peat may allow medium to retain excessive water, which probably brought anaerobic condition and stressed adventitious root formation. The addition of Bora-tsuchi (coarse particles) to the peat moss possibly improved aeration and retained water in a balance other than to over saturate the substrate. This was also confirmed by Al-Salem and Karam (2001) who improved rooting of the Greek strawberry by increasing perlite to the peat moss medium.

Our result of wounding plus IBA treatment was similar to that of Howard et al. (1984), who found a significant rooting response of the winter cuttings of apple rootstock M.26 to wounding and IBA treatment. Hardwood cuttings of the bitter almond collected in November also exhibited significantly maximum rooting and number of roots when wounding preceded IBA 10,000 ppm solution (Kasim et al., 2009). Wounding by making two opposite longitudinal incisions at the base of the cuttings and using different IBA concentrations were significantly effective for *Arbutus andrachne* L. root growth (Al-Salem and Karam, 2001). This paper later concluded that the rooting percentage was three times greater in the wounded cuttings than in the non-wounded. A threaten plant species *Leucadendron discolor* E. Phillips & S. Hutch, though to be difficult to propagate by stem cuttings, showed the high rooting percentages in the both terminal (85%) and basal (52.5%) stem cuttings when wounding followed with 4000 ppm IBA solution (Rodríguez Pérez et al., 1997). IBA without wounding encouraged less than 30% rooting in all the concentrations (Rodríguez Pérez et al., 1997). Apart from in vivo, in vitro rooting of a promising pear rootstock (*Pyrus betulaefolia* L.) was also increased with wounding followed by the low concentration of IBA (Pasqual et al., 2002).

Based on the result we obtained from a preliminary trial in 2015, 0.5% IBA in talc, without wounding didn't have stimulatory effects on adventitious root formation of 'MKR1' unmounded cuttings. Tetsumura et al. (2001a) also reported no impact of IBA to both mounded and unmounded hardwood sucker cuttings, soaked in 25 ppm IBA for 24 h or dipped in 3000 ppm IBA for 5 s. They stated that a possible reason for the ineffectiveness of the IBA treatment might be degradation of the IBA before it could exert an effect. In contrast, our study showed significant improvement to the wounding plus IBA treatment. Wounding might expose more cambium to the IBA and talc might avoid the rapid degradation. MacKenzie et al. (1986) illustrated that the incision as wounding treatment induced new cambium differentiates within the callus from undamaged cambium on either side. The new cambium then was sensitive to the applied auxin. Thus, high callusing (79%) of the wounded treatment of 'MKR1' seemed to be the case for IBA effectiveness (Table 2). 'Jiro' achieved the best rooting without wounding and IBA treatment. On the other hand, the high callusing (33.3%) of 'MKR1' with poor rooting (8.3%) might be due to the lack of exogenous auxin application (Table 1). Rooting via callus formation may depend on genotypes. A significant yield of the shoot fresh, dry weights, leaf number and leaf area by wounding in our study could be related to increased water absorption. Lin et al. (1997) described increase in water uptake as wounding benefit which keeps cutting leaves turgid.

In the present study, improvement in adventitious root formation of 'MKR1' would be more due to callus sensitivity to the applied auxin. Hence, stimulating callus with different types of wounding with or without IBA is required to be tested in the future researches.

In conclusion, although our results indicated that mounding alone was effective for 'Jiro' rooting, the addition of wounding and IBA would be necessary to stimulate rooting in 'MKR1' hardwood cuttings. Instead of pure peat, two-component medium of the peat and bora-tsuchi might be suggested for the planting.

Experiment 2

Propagation from single node softwood cuttings is now a practical method for obtaining clones of several persimmons. Cuttings planted in late June were more suitable for this method of propagation and responded more favorably to the substrate than did cuttings in late July or August (Table 3). Our results agreed with several reports that showed time of collection had a great influence over rooting of cutting of some trees and shrubs (Jalil and Sharpe 1956; Ryan and Frolich, 1962; Tetsumura et al., 2000; Pijut and Moore, 2002).

Tetsumura et al. (2000) reported that single node stem cuttings of a dwarfing rootstock of the Japanese persimmon (*Diospyros kaki* L.) produced high percent rooting and survival either in late June and July, while significantly low rooting and survival was occurring from the cuttings planted in August. Similarly, cuttings prepared from the root-suckers of 'Nishimurawase' and 'Jiro' rooted better when set in June than in July or August (Tetsumura et al., 2000). The rooting response of basal cuttings of 'Bartlett' pear was as high in June (100%) as it did in March (Ryan and Frolich, 1962). However, cuttings taken in May 23 did not root (Ryan and Frolich, 1962). Rooting percentage and quality of the peach cuttings taken in June were best, but gradually became poorer in July and August (Jalil and Sharpe, 1956). Gur et al. (1974) tested leaf-bud cuttings (including a small branch piece) for the almond × peach hybrid under intermittent misting. Success only achieved in May or in June planting. In our study, less or few rooting of the late July and late August planting may be due to a reduction in the growth rate of the suckers. Tetsumura et al. (2000) also linked decreases in rooting ability with growth rate of the root-suckers. The cuttings of deciduous shrub (*Amelanchier alnifolia* Nutt), with edible berry-like fruit, taken in late June returned better rooting than those taken 2 weeks earlier (Bishop and Nelson, 1980). The authors added that time and stage of growth were the most important considerations in regard to Saskatoon (*A. alnifolia* Nutt) propagation. Compared to August, June was the best season for clonal multiplication of *Alnus maritima* (Marsh.) Muhl. ex Nutt. landscape woody-plant species (Schrader and Graves, 2000). When means of the cuttings sources and IBA treatments were combined, more cuttings rooted (41%), survived (72%), and callused (67%) in June than did cuttings collected in August (8% rooted, 31% survived, 29% developed callus). In a correlation with our study where June yielded a significantly high root length, *A. maritima* also developed longer roots in June than did cuttings taken in August (Schrader and Graves, 2000). Pijut and Moore (2002) suggested June with 62 mM K-IBA or 74 mM IBA for the greatest rooting success (76.9 and 87.5%) of the butternut softwood cuttings. Japanese chestnut (*Castanea crenata* Sieb. et Zucc.) like Japanese persimmon is not easy to root (Tetsumura et al., 2008). However, the optimum rooting was evident for June (90%) and July (98%) than cuttings inserted in August (71%). Based on visual inspections, weather was rainy during late June planting, however, it was hot and sunny during late July and August. Hence, leaves desiccation which caused low rooting and survival in the latter months might be related to maximum ambient heating.

There was no significant difference between FTP and mist for rooting and survival (Table 3). Goh et al. (1995) also observed no significant difference between open and closed propagation chamber regarding survival and rooting percentages of *Gymnostoma sumatranum* [syn. *Casuarina sumatrana* (de Vriese) L.] softwood cuttings. He explained open chamber as a perforated plastic trough with cuttings put in a greenhouse, covered with a transparent polyethylene film and supported by a metal frame. Likewise, cuttings in a close chamber were tightly covered by Neoflon film, maintained high humidity around stem cuttings without any gas or temperature built up. Çet al. (1994) revealed that cutting of *Olea europaea* 'Gemlik' planted in the end June rooted better under shaded polyethylene tunnel (SPT) than in mist. SPT which was covered by a transparent polyethylene sheet and shaded with coarse white calico obtained overall 30-100% rooting, while mist did 0-40%. An economic tree of Shea (*Vitellaria paradoxa* Gaertn) acting as source of the income for semi-arid west Africa, multiplied successfully through a propagation bin (63.3%) and polyethylene propagators (57.5%) (Yeboah et al., 2011).

It is well known that nutrient leaching takes place from cuttings and often leaves become chlorotic under mist (Preece, 2003). Despite leaching problems, we observed deep humidity fluctuations to the mist during day-night period. Hence, significant difference for the FTP in terms of June rooting could be associated to consistence humidity and retention of the essential soluble plant substances. Contact tent polyethylene enclosures were also more favorable in relation to better rooting of three ornamentals than either of open propagation or mist (Mudge et al., 1995). Goh et al. (1995) concluded that a closed chamber without mist seemed to be an efficient plant propagation system, which required little attention and induced high yield. Mudge et al. (1995) revealed that earlier stomatal closure may have occurred in response to the higher temperature, which in turn reduced further water loss from contact polyethylene-enclosed cuttings. This phenomenon probably would be the case for the effectiveness of the FTP in our study.

The result displayed significantly lower callus for the FTP than was in the mist (Table 3). Tetsumura et al. (2008) also obtained high callus of the Japanese chestnut to the mist and fewer with the micro mist. They noted that formation of the callus might be affected by a propagation environment as well as a genotype of the cutting. Although, the cuttings were prepared from a single genotype, high callus of the mist in our study could be relatrd to the environment. In addition, we speculate that significantly high mean root length of the FPT would be due to water status. We have noticed that roots were growing well out of the drainage holes; however, they were not evident in the mist as pots were placed on a raised bench.

Rooting percentage, root length, and callus were affected by propagation substrates (Table 3). Peat produced the highest percent rooting, but it was significantly lower in perlite. The softwood cuttings of the red raspberry also rooted well and had more fibrous roots in peat than that of perlite, which didn't induce roots at all (Kobayashi et al., 1999). Under mist propagator, Jalil and Sharpe (1956) found inferior rooting of the peach cuttings in the grade 8 (2-3 mm) perlite. He asserted that perlite was the coarsest substrate and may hold less water than any of the other substrates. Mixing coarse perlite with peat and either used fine perlite alone returned significantly higher rooting (Jalil and Sharpe, 1956). Tetsumura et al. (2009) also observed cuttings of the Japanese chestnut in Bora-tsuchi easily wilted and died earlier in the mist than those in Metro-Mix®. They thought it might be associated with high porosity of Bora-tsuchi, where cuttings without roots couldn't uptake enough water from the cut surface. However, in a parallel experiment, Bora-tsuchi showed improved rooting to the micro mist, because of maintaining more than 60% relative humidity at midday even in the dry hot summer (Tetsumura et al., 2009). In a similar manner, perlite returned less rooting in the mist, but, it remarkably enhanced rooting with the use of FTP (Table 4). The fact that FTP contained always plenty of water at the bottom of the tray, which may replace transpired water rapidly and kept the ambient humidity optimum even in hot mid-days. Besides, good water holding capacity of the Metro-Mix® probably reflected the longest root length for this treatment.

Although, perlite was less responsive in some studies, it was more positive to the rooting of softwood cuttings of some woody species (Aiello and Graves, 1998; Fatemeh and Zaynab, 2014; Graves and Zhang, 1996; Jalil and Sharpe, 1956; Prat et al., 1998). Prat et al. (1998) reported that perlite alone was as good as its mixtures for the rooting of jojoba clones. It also exhibited better percent rooting, number of roots, root length, fresh and dry weights of the *Schefflera* cuttings than a couple of other substrates (Fatemeh and Zaynab, 2014). Kobayashi et al. (1999) insisted that a well-drained medium is important to select, because red raspberry cuttings were sensitive to overwinter. Thus, significantly high callus and 100% acclimatization survival in perlite might be attributed to a well drain status of the medium. Conversely, poor rooting of the perlite could be referred to the rapid loss of the water (Tables 3 and 4).

Researchers mostly focus to reduce dead cuttings and increase root formation, while few of them considered acclimatization. A special care should be taken for the hardening, because most of the plants are dying in this stage, particularly after transplanting into another or fresh medium. During hardening of our experiments, few cuttings were yellowed shortly after being weaned from the mist bench. Although there was no correlation between root number and cutting survival, desiccation often has been evident to the cutting which had one and short root. Perhaps, cuttings with only one root may easily die even from slight damages because an alternative root doesn’t exist. Tetsumura et al. (2000) also considered abundant roots important for successful transplanting.

Although, perlite and Metro-Mix® maintained 100% percent acclimation survivals, they have shown deficiencies-like symptoms to the cutting foliage in late growth. These symptoms were also displayed by vermiculite with the exception of peat, which was the only intact treatment. Gur et al. (1974) found vermiculite suitable for a short time; however, for a long time growing of the rooted cuttings, he suggested mixture of the perlite and peat.

As a conclusion, FPT can utilize a number of substrates with maximum rooting than that of intermittent misting. It would be a safe substitute for the mist; however, additional research is needed to evaluate the use of FPT in large-based production. This may lower costs of the saplings and reduce the energy expenses.

Experiment 3

IBA concentrations significantly affected most of the rooting traits (Table 5). Percent rooting increased with increases in IBA concentration up to an optimum, before decreasing steadily. The number of roots per cutting and root lengths sharply increased at high concentrations. Root fresh and dry weights significantly increased from lower to high concentrations (Table 5). A similar trend was evident for the pomegranate semi-hardwood cuttings, where 9000 ppm was significantly an optimum for all the rooting characteristics, before falling at 12,000 ppm IBA (Owais, 2010). Olive stem cuttings also showed significantly maximum rooting (60%) at the first IBA treatment (3000 ppm) than it did either the control or high concentration (Kurd et al., 2010). Aminah et al. (1995) reported that IBA application improved number of roots on each cutting, but, higher doses were less responsive to the rooting of the *Shorea leprosula* single node leafy cuttings.

As a conclusion, although most of the rooting parameters except percent rooting gradually increased with increases of IBA, high concentration in particular 4000 ppm and 5000 ppm were appeared detrimental to tender bases of the ‘MKR1’ softwood cuttings.

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