Application of Floating Culture System on Clonal Propagation of Taiwanese Wild Grape

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Additional index words. *Vitis thunbergii*, softwood cuttings, rooting, circulating water, propagation medium temperature

Summary. **In this study, we built a floating culture system, which could improve the rooting percentage of stem cuttings of taiwanese wild grape (*Vitis thunbergii*). We took softwood cuttings instead of hardwood cuttings and tested the effects of cutting type, medium type, and auxin concentration on the rooting percentage. In the first experiment, single leaf cuttings (SLC) in 26 °C circulating water (CW_{26}) produced 82% rooting as compared with 48% rooting in the subirrigation medium [SM (3 horticultural perlite : 2 peatmoss, by volume)]. The highest rooting percentage of 88% occurred in 30 °C circulating water (CW_{30}). The same trend was also observed in terminal cuttings (TC) with 62% rooting in CW_{26} compared with 27% in SM. The highest rooting percentage of 83% occurred in CW_{30}. Besides having the highest rooting percentage, TC and SLC in CW_{30} formed adventitious roots 7 and 9 d earlier than in 22 °C circulating water (CW_{22}). In the second experiment, SLC in 1.25 μM 1-naphthalenacetic acid (NAA) solution produced 92% rooting as compared with 82% rooting in the untreated group. In addition, SLC in all NAA solution treatments formed adventitious root 6 d earlier than in the untreated group. Based on these results, we suggest that the floating culture system is a practicable system for the clonal propagation of taiwanese wild grape.**

Taiwanese wild grape is a perennial herb that is traditionally used in medical therapy in eastern Asia. Recently, growers tried to cultivate taiwanese wild grape artificially, but they had some problems such as low rooting percentage in clonal propagation. In a preliminary study in 2003, we examined the rooting percentage of clonal hardwood cuttings of taiwanese wild grape using conventional materials. However, the rooting percentage of taiwanese wild grape hardwood cutting was too low to be useful for mass propagation. It was reported that taiwanese wild grape could be clonal propagated through high-frequency shoot tip culture (Lu, 2005). However, the proliferation system takes lots of labor and time. Some reports mentioned that softwood cuttings could be used for clonal propagation of norton grapes (*Vitis aestivalis*) and muscadine grapes (*Vitis rotundifolia*) (Goode and Lane, 1983; Keeley et al., 2003). So we tried to take softwood cuttings instead of hardwood cuttings for clonal propagation.

For softwood cuttings, many researchers have focused on the atmospheric environment of the propagation area to promote rooting percentage. Since the 1950s, overhead intermittent mist irrigation has been the conventional means of maintaining the water potential of cuttings and it has increased the rooting percentage in many plant species (LeBude et al., 2004; Mudge et al., 1995). Subirrigation is another common system used to reduce water stress for successful rooting of cuttings (Owen et al., 2003; Post, 1946; Zhang et al., 1997). However, these two systems are costly in terms of equipment. Therefore, we built a floating culture system (Fig. 1) for clonal propagation of taiwanese wild grape.

The objective of this study was to examine the effects of cutting type, medium type, and auxin concentration on the rooting percentage of taiwanese wild grape in a floating culture system.

Materials and methods

Plant materials. Ten clonally propagated vines of taiwanese wild grape (4 years old) were cultivated in the vineyard of the department of horticulture at National Taiwan University (Taipei, Taiwan). Softwood cuttings were collected on 11 July (for the first experiment) and 10 Aug. (for the second experiment) in 2005. After collection, each cutting was immediately inserted into water. Cuttings were transported to the laboratory on the collection day and were divided into two cutting types as follows: 1) terminal cuttings, terminal bud with four young leaves and five nodes (≈10 to 12 cm in length); or 2) single leaf cuttings, latent axillary bud with one mature leaf, and one node (the sixth to 10th node from the terminal bud, ≈5 to 7 cm in length).

Experimental conditions. Experiments were conducted in a controlled-environment growth chamber in the laboratory. The chamber setting was 25 °C day/20 °C night and 90% relative humidity. Light (average 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was applied by fluorescent lamps 20 to 25 cm above the propagation canopy 16 h each day. The floating culture system (Fig. 1) was composed of a water bath circulator (B206-T2; Firsteck Scientific, Taipei, Taiwan) and floating microtube racks (MF16; Bioman Scientific, Taipei, Taiwan). The initial water volume of each system was 12 L. Cuttings were put into the holes of floating racks and inserted at a depth of ≈4 cm in distilled water. Distilled water was added, as needed, directly into the tank to maintain the water volume.

Medium treatments and experimental design. In the first experiment, four different media were examined: 1) subirrigation medium, which was a mixture of 3 perlite : 2 peatmoss (by volume); 2) 22 °C circulating distilled water; 3) 26 °C circulating distilled water; or 4) 30 °C circulating distilled water. The water

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Units

To convert U.S. to SI, multiply by	U.S unit	SI unit	To convert SI to U.S., multiply by
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
(°F – 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

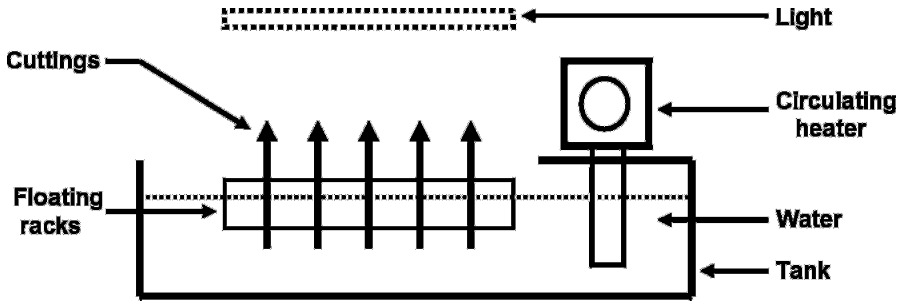

Fig. 1. Diagram of the floating culture system used for clonal propagation of taiwanese wild grape. It was composed of a water bath circulator and floating microtube racks. Cuttings were put into the holes of floating racks and inserted in the distilled water. Light (average 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was applied by fluorescent lamps 20 cm (7.9 inches) above propagation canopy 16 h each day.

media were circulated and heated by water bath circulators. The subirrigation medium (SM) was filled in plastic baskets (40 cm long, 25 cm wide, 5 cm high) with drain holes toward the bottom. To maintain the water potential of SM, all baskets were set in plastic trays (45 cm long, 30 cm wide, 1 cm high) with 1-cm deep distilled water. Water checking was done twice each day to make sure that the water level of SM was maintained at 1 cm deep. The temperature of SM was measured as 26 ± 1 °C. We measured the rooting percentage, root number, and the total length of roots 3 weeks after cutting. A cutting was considered rooted if it had at least one root greater than 2 mm in length. To compare the adventitious root formation rate of cuttings in different media, we recorded the rooting percentage of cuttings in circulating water (CW) everyday (to avoid hurting the roots of cuttings in SM, we only observed them at the end of this experiment) and calculated the day on which 50% rooting of cuttings were rooted (R_{50}).

In the second experiment, 1-naphthalenacetic acid (NAA) was added to the CW_{26} at the following rates: 1.25, 2.5, and 5 μM NAA solutions held at 26 °C were applied as rooting media. CW_{26} was used as a control. We recorded the rooting percentage everyday and calculated the day on which R_{50} occurred. This experiment was conducted for 3 weeks.

A completely randomized design was used for both experiments. One hundred cuttings [50 terminal cuttings (TC) and 50 single leaf cuttings (SLC)] were used per floating culture system. Each treatment involved three replications for a total of 12 floating culture systems used per experiment.

STATISTICAL ANALYSIS. The mean values, SDs, and statistical differences were evaluated through an analysis of variance that was performed with SAS (v8; SAS Institute, Cary, NC). The main effects of the cutting type, the medium type, and the two-way interaction were tested for all rooting traits assessed. To homogenize the variances between different treatments, the variables of rooting percentage were transformed by using the arcsine of the square root.

Results and discussion

In SM, SLC had 48% rooting compared with 27% for TC. The same trend was also observed in circulating water media such as CW_{22} and CW_{26} media with SLC having a higher rooting percentage than TC. However, there was no difference in rooting percentage between SLC and TC in CW_{30} medium (Table 1). For softwood cuttings, wilting and rot are lethal factors in the first few days after being cut from the vines. SLC are more mature and usually have more lignified tissues than TC and can maintain tissue water potential more constantly and have greater chances to form adventitious root (Goode and Lane, 1983). We assume that TC in CW_{30} treatment formed adventitious roots earlier and thus the wilting possibility was dramatically reduced. Therefore, the rooting percentage of TC in CW_{30} increased and showed no statistical difference from SLC.

Cuttings in circulating water rooted significantly better than in SM and the rooting percentage was greatest in the CW_{30} medium than in CW_{26} or CW_{22} media (Table 1). This may be because cuttings in water can maintain tissue water potential more constantly than in SM and have greater chances to form adventitious root. Mixtures of sand or perlite with peatmoss are widely used as rooting media for cuttings because these mixtures can hold water, which prevents the lower end of the stem from drying while remaining loose enough to allow oxygen to reach the newly forming roots (Sabir et al., 2004). Water is not thought to be a good medium to root most cuttings because an adequate amount of oxygen cannot reach developing roots (Soffer and Burger, 1988; Zimmerman, 1930). To increase the concentration of oxygen in water medium, bubbling was reported as an applicable method (Zimmerman, 1930). Water culture was suggested as a simple and efficient method for rooting some species' woody cuttings (Komissarov, 1968). In this study, circulating water was a suitable medium for rooting softwood cuttings of taiwanese wild grape.

For TC, the R_{50} occurred on the 13th day after cutting in CW_{22}. When we increased the medium temperature, the time required to reach R_{50} was antedated to the eighth day and sixth day in CW_{26} and CW_{30}, respectively (Table 1). The same trend was also observed in SLC with R_{50} occurring 9 d earlier because of heating the circulating water to 30 °C rather than 22 °C (Table 1). In addition, the total length of roots of TC and SLC in CW_{30} were 19.7 and 13.9 cm, respectively, as compared with 7.8 and 8.8 cm in CW_{26} (Table 1). We suspect that heating the medium temperature to 30 °C accelerated the metabolism rate as well as the adventitious root formation process.

Similar experiments were also reported in recirculating subirrigation systems used for softwood propagation of sparkleberry holly (*Ilex verticillata*) (Owen et al., 2003) and maple (*Acer rubrum*) cuttings (Zhang et al., 1997) with perlite medium. For sparkleberry holly, rooting percentage of cuttings increased from 38% to 80% while heating media from 20 to 23 °C. This result

Table 1. Traits assessed in the medium type experiment of softwood cuttings of taiwanese wild grape.

Medium type[z]	Rooting (%)		Roots per cutting (no.)		Total length of roots (cm)[z]		Time to 50% rooting (d)	
	Single leaf cuttings	Terminal cuttings	Single leaf cuttings	Terminal cuttings	Single leaf cuttings	Terminal cuttings	Single leaf cuttings	Terminal cuttings
Subirrigation medium (SM)	48 ± 4 d[y]	27 ± 4 d	3.0 ± 0.2 a	3.3 ± 0.3 b	8.3 ± 0.6 b	7.0 ± 1.2 b	—[x]	—
22 °C circulating water (CW_{22})	61 ± 3 c	52 ± 3 c	2.5 ± 0.2 b	3.0 ± 0.3 b	6.1 ± 0.5 c	6.4 ± 0.7 b	16 ± 1 a	13 ± 1 a
26 °C circulating water (CW_{26})	82 ± 2 b	62 ± 3 b	2.5 ± 0.2 b	3.0 ± 0.2 b	8.8 ± 0.4 b	7.8 ± 0.6 b	13 ± 1 b	8 ± 1 b
30 °C circulating water (CW_{30})	88 ± 2 a	83 ± 3 a	2.9 ± 0.1 a	3.5 ± 0.2 a	13.9 ± 0.9 a	19.7 ± 2.6 a	7 ± 1 c	6 ± 1 c
Source of variation	**df**							
Cutting (C)	1	35.52**[w]		3.37 NS		35.52**		
Medium (M)	3	8.28*		103.71**		8.28*		
C × M	3	0.34 NS		12.65**		0.34 NS		

[z](1.8 × °C) + 32 = °F; 1 cm = 0.3937 inch.
[y]Means with different letters with a column indicate significant differences ($P < 0.05$) by Duncan's multiple range test.
[x]Not measured.
[w]F values with indicated level of significance are given for each trait. NS,*,**Nonsignificant or significant at $P < 0.01$ or 0.001, respectively.

provided more evidence that heating medium may help rooting percentage of softwood propagation. However, medium heating above 30 °C will decrease the rooting percentage for maple cuttings. This may be caused by the different plant species and overheating the perlite medium. Burholt and Van't Hof (1971) reported that root formation, which resulted from cell differentiation and elongation, is a temperature-regulated process with an optimum of ≈25 °C in sunflower (*Helianthus annuus*). Dykeman (1976) reported that optimal medium temperatures differed for root initiation and elongation, and these temperature optima also varied among species. Thus, in the application of the floating culture system, plant provenance and genotype should be considered when considering optimum water temperatures.

Applications of exogenous auxin also were effective in promoting adventitious root formation in grapes (*Vitis* spp.) (Keeley et al., 2003; Lu, 2005; Singh et al., 2004). We observed that the rooting percentage of SLC increased to 92% when we added 1.25 μM NAA, but the rooting percentage at 2.5 μM NAA medium was similar to the untreated control (Fig. 2). When we used 5 μM NAA medium, the rooting percentage decreased to 78%. This result shows that a low concentration of auxin could increase the rooting percentage. NAA also appeared to shorten the adventitious root formation time of SLC. R_{50} occurred around the sixth day for all NAA treatments, which was 6 d earlier than the untreated control (Fig. 2). TC in NAA solutions all wilted and rotted on the third to fifth day after treatments (data not shown). A similar phenomenon was reported in the vegetative propagation of eastern hemlock (*Tsuga canadensis*) softwood cuttings (Robert et al., 2005). The possible explanation is chemical toxicity.

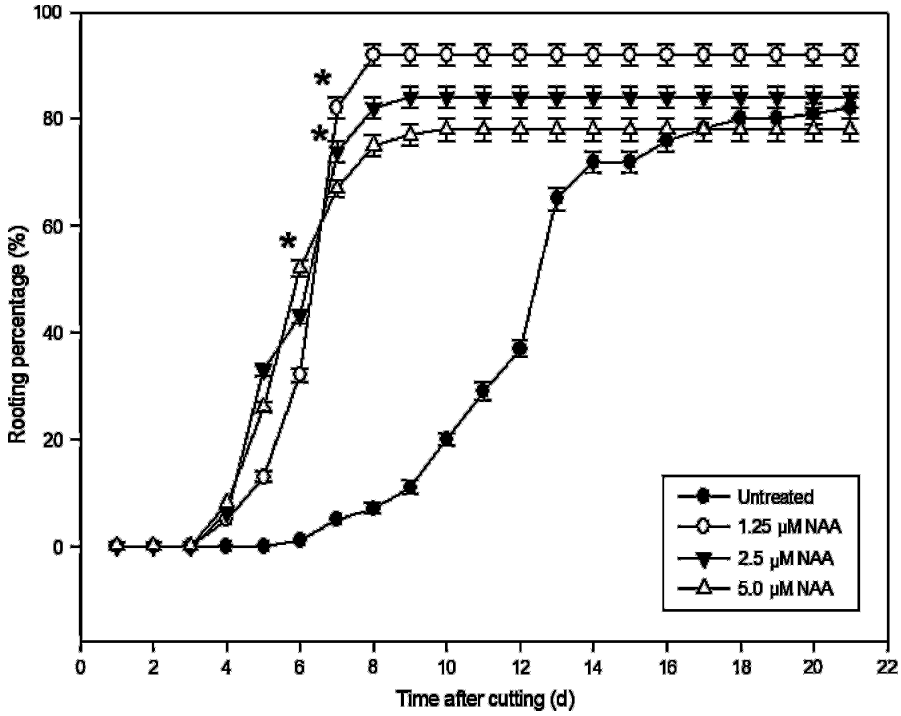

Fig. 2. Effects of 1-naphthalenacetic acid (NAA) solution concentration on rooting percentage of single leaf cuttings of taiwanese wild grape in the floating culture system. Distilled water was used as an untreated control. The temperature of solution was 26 °C (78.8 °F). The bars indicate SE. *Significant at $P < 0.05$.

This research demonstrates that the floating culture system could be used for clonal propagation of taiwanese wild grape in a controlled environment and that 30 °C circulating water is a sufficient medium. Softwood cuttings appear to be good starting materials for clonal propagation, especially for species that cannot form adventitious roots well by hardwood cuttings such as taiwanese wild grape.

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