Effects of Various Conditions on Root and Emergent Shoot Growth during Propagation by Cuttings in the Amenity Plant *Hardenbergia violacea*©

Yasuhiko Koike, Atsuhiko Yamazaki, Yozo Mitarai and Ryo Norikoshi
Faculty of Agriculture, Tokyo University of Agriculture, 1737 Funako, Atsugi, Kanagawa, 243-0034, Japan
Email: koike@nodai.ac.jp

To develop a mass propagation method for *Hardenbergia violacea* L., new shoots were prepared and used to compare the effects of rooting medium, temperature, photoperiod and concentrations of IBA (3-indolebutyric acid) and surfactant on rooting. The upper nodes of one-node cuttings bearing two leaves were cut half and placed in 16 ppm IBA solution for 1 h. The cuttings were then placed in rockwool cubes and maintained under 16-h photoperiod at 20°C on 4 weeks from the time of cutting to assess the extent of root formation. High percentages of rooting were observed, with the highest observed in the solution containing IBA and 0.05% surfactant.

INTRODUCTION

In open spaces and parks, and in public and private grounds, flowers, and other greenery are being grown to produce a congenial environment for human activities. Flowering plants reared for such purposes are know as amenity flowers (Imanishi, 2000). Recently, these have also been called amenity plants. In today's overcrowded cities where urban landscaping counts, beautification of the environment and the creation of amenity values has become an important consideration.

Amenity plants and flowers not only provide a sense of peace, naturalness, and pleasant along with greenery but also create amenities and urban landscaping. As such, the role of amenity plants should continue to grow in importance.

One promising candidate for an amenity plant is *Hardenbergia violacea* L., an evergreen vine in the legume family originating from Eastern Australia (Australian Native Plants Society, 2012). This plant has thick elongated triangular leaves of dark green. Each flower spike contains tens of florets. It is easy to cultivate and has fairly good cold resistance, making it suitable as an amenity plant.

Flowering plants can be propagated by vegetative propagation methods that take advantage of the ability of the plant organs to regenerate. This produces new plants with the same trait as the parent. Among these, cutting propagation is a promising method where cuttings can be taken from leaves, stems, and roots, and placed in a suitable soil medium to obtain new plants from adventitious shoots and roots (Imanishi, 2000). It does not need any advanced technology, the propagation rate is quite high, and the method is easy to incorporate into existing cultivation methods.

In this experiment our laboratory tested some simple but promising cuttings propagation methods for propagating *H. violacea* with various conditions. The purpose was to contribute towards amenity planting with *H. violacea*.

MATERIALS AND METHODS

Three-year-old *H. violacea* stock was used for this experiment. The experiment was run from 5 April to 5 May. Forty fully grown plants were used. Branches from the current year were collected, and cuttings were taken from sections of the upper shoots to the lower part of a leaf pair. They were made up of a section of stem about 5 cm.

All cuttings except those used for experiments on rooting acceleration agents were soaked for 60 min with 16 ppm indole-3-butyric acid (IBA) in Oxyberon solution (Bayer Crop Science Co. Ltd.), then planted in seeding boxes (length 42 cm, width 32 cm, depth 8 cm) at intervals of 10 cm.

For all experiments except those testing the effect of photoperiod, the plants were maintained in an incubator (MIR-553, Sanyo Electrical Co. Ltd) at a constant temperature and under LD 16:8 conditions with 15W fluorescent lighting (10 µmol·$m^{-2}·s^{-1}$). Twenty samples were used for each experimental group. Four weeks after cuttings were taken; the rate of rooting, the numbers of roots per rooted shoot, the shoot lengths and the number of nodes were recorded.

The Effect of Growing Media on Root Development and Shoot Lengths
The media tested were: mixture of sand, peat moss, and akadama soil (conventional soil) (2:2:1, by vol.); rock wool (40×35×35 mm, Nitto Boseki Co. Ltd.); or perlite. The cuttings were cultivated at a constant temperature of 20°C under 16 h light. Four weeks after the cuttings were taken; the rate of rooting, the numbers of roots per rooted shoot, the shoot lengths, and the number of nodes were recorded.

The Effect of Temperature on Root Development and Shoot Lengths
Cuttings were cultivated in rock wool and kept in an incubator at temperatures of 10, 15, and 20°C under LD 16:8 conditions.

The Effect of Photoperiod on Root Development and Shoot Lengths
Cuttings were cultivated in rock wool and kept in an incubator at a constant temperature of 20°C and photoperiods of 10, 12, and 16 h light under a fluorescent light (10 µmol·$m^{-2}·s^{-1}$).

The Effect of Different Concentrations of Rooting Accelerant on Root Development and Shoot Lengths
Cuttings were immersed in Oxyberon solution at concentrations of 0, 8, 16, 40, and 80 ppm IBA, and the cuttings were cultured in rock wool at 20°C and LD 16:8.

The Effect of Rooting Accelerant and Surfactants on Root Development and Shoot Lengths. Cuttings were immersed in 16 ppm IBA Oxyberon solution combined with 0-0.3% of the surfactant Tween 20 (Kanto Chemical Co. Ltd). The cuttings were cultivated in rock wool, at 20°C and LD 16:8.

RESULTS AND DISCUSSION

The Effect of Growing Media on Root Development and Shoot Lengths
Adventitious roots were observed 10 days after cuttings were taken, regardless of the growing medium. The rate of rooting was 100% in the conventional soil, rock wool, and perlite (Table 1). The number of roots was higher in the rock wool than the other two media. No difference in shoot length or number of nodes with growing media was observed.

The Effect of Temperature on Root Development and Shoot Lengths
Maximum root development was observed at 20°C, followed by the 15°C treatment. No root development was observed at 10°C (Table 2). The number of roots, shoot lengths, and number of nodes were significantly higher under the 20°C treatment.

The Effect of Photoperiod on Root Development and Shoot Lengths
The rate of rooting was higher under LD 16:8 than under LD 10:14 or LD 12:12. The number of roots per rooted shoot was highest under LD 16:8 conditions. The shoot length and number of nodes were both highest under LD 16:8 conditions (Table 3).

Table 1. Effect of rooting medium on rooting and shoot growth of cutting in *Hardenbergia violacea* L.

Medium	Rate of rooting (%)	No. of roots per rooted shoots^z	Length of the shoot (cm)	No. of nodes
Soil	100a	8.0c^y	5.3a	3.3a
Rockwool	100a	10.0a	5.5a	2.9a
Perlite	100a	9.2b	5.2a	3.0a

After cutting, plants were subjected to 16-h photoperiod in a growth chamber kept at 20°C. Observations were carried out 4 weeks after cutting. 20 cuttings were used in each treatment.
^{z}More than 1 mm in length.
^{y}Mean separation within the column by Tukey's HSD test, 5% level of significance.

Table 2. Effect of temperature on rooting and shoot growth of cutting in *Hardenbergia violacea* L.

Temperature (°C)	Rate of rooting (%)	No. of roots per rooted shoots^z	Length of the shoot (cm)	No. of nodes
10	0c	-	-	-
15	80b	8.2b^y	5.0b	2.9b
20	100a	9.8a	5.5a	3.2a

After cutting, plants were subjected to 16-h photoperiod in a growth chamber kept at various temperature. Observations were carried out 4 weeks after cutting. 20 cuttings were used in each treatment.
^{z}More than 1 mm in length.
^{y}Mean separation within the column by Tukey's HSD test, 5% level of significance.

Table 3. Effect of photoperiod on rooting and shoot growth of cutting in *Hardenbergia violacea* L.

Photoperiod (h)	Rate of rooting (%)	No. of roots per rooted shoots^z	Length of the shoot (cm)	No. of nodes
10	45b	2.0c^y	1.5c	1.8c
12	95a	5.5b	4.8b	2.4b
16	95a	7.5a	5.6a	3.6a

After cutting, plants were subjected to 10, 12, or 16-h photoperiod in a growth chamber kept at 20°C. Observations were carried out 4 weeks after cutting. 20 cuttings were used in each treatment.
^{z}More than 1 mm in length.
^{y}Mean separation within the column by Tukey's HSD test, 5% level of significance.

The Effect of Different Concentrations of Rooting Accelerant Oxyberon Solution on Root Development and Shoot Lengths

The control treatment (0 ppm) resulted in a rooting rate of 10% (Table 4). When 16, 40, and 80 ppm IBA had been used, the rooting rate was 100%. The number of roots was significantly higher under the 16, 40, and 80 ppm treatments when compared with the other treatments, as were the shoot lengths and number of nodes.

Table 4. Effect of IBA concentrations on rooting and shoot growth of cuting in *Hardenbergia violacea* L.

IBA concentrations (ppm)	Rate of rooting (%)	No. of roots per rooted shoots^{z}	Length of the shoot (cm)	No. of nodes
0	10d	-	-	-
4	30c	2.8d^{y}	4.3d	2.6d
8	50b	3.5c	4.7c	3.1c
16	100a	8.3a	6.1a	3.5a
40	100a	8.5a	6.2a	3.4a
80	100a	8.6a	6.2a	3.5a

Cuttings were soaked in different IBA solution for 60 min before planting into rockwool. After cutting, plants were subjected to 16-h photoperiod in a growth chamber kept at 20°C. Observations carried out 4 weeks after cutting. 20 cuttings were used in each treatment.
^{z}More than 1 mm in length.
yMean separation within the column by Tukey's HSD Test, 5% level of significance.

The Effect of Rooting Accelerant and Surfactants on Root Development and Shoot Lengths

When cuttings were treated with a surfactant alone, no root development was observed throughout the experiment (Table 5). When 0.05% surfactant was added to 16 ppm IBA Oxyberon solution, the root development rate was 100%. This also caused a significant increase in shoot length and number of nodes.

To be its most effective, soil used for reproducing by cuttings needs to be porous, have moderate water-holding capacity, and good drainage. It should also not include fertilizer or organic matter, and should be clean (Fujii, 1968).

Table 5. Effect of the combination treatment of IBA and surfactant concentrations on rooting and shoot growth of cutting in *Hardenbergia violacea* L.

IBA concentrations (ppm)	Surfactant concentrations (%)	Rate of rooting (%)	No. of roots per rooted shoots^{z}	Length of the shoot (cm)	No. of nodes
0	0	0	-	-	-
	0.05	0	-	-	-
	0.1	0	-	-	-
	0.2	0	-	-	-
16	0	90b	8.3d^{y}	4.6c	1.2c
	0.05	100a	11.5a	6.8a	3.3a
	0.1	95b	9.8b	5.1b	2.8b
	0.2	80c	9.1c	3.8d	1.3c

Cuttings were soaked in combination of IBA and surfactant solution for 60 minute before planting into rockwool. After cutting, plants were subjected to 16-hr photoperiod in a growth chamber kept at 20°C. Observations were carried out 4 weeks after cutting. 20 cuttings were used in each treatment.
^{z}More than 1 mm in length.
^{y}Mean separation within the column by Tukey's HSD test, 5% level of significance.

In ornamental trees, any differences in the physical properties of the rooting medium are reflected in variations in root development rate (Tilt and Bilderback, 1987). In addition, the higher the moisture content, the higher the rate of rooting (Rein et al., 1991). In this experiment, no difference between the rate of rooting, number of roots per rooted stem, shoot length, or number of nodes was found when cuttings were grown on the standard sand; peat moss; akadama mixture, rock wool, or perlite (2:2:1, by vol.) (Table 1). This shows that in *H. violacea*, any medium that is porous, retains water, and drains well is suitable for cuttings.

The suitable temperature for propagation by cuttings depends on the plant but usually ranges from 15-25°C (Fujihara, 1984). In *Dorycnium*, Alegre et al. (1998) report higher rooting rates in cuttings reared at 20°C when compared to cutting reared in a greenhouse with a minimum temperature of 10°C. In this experiment, all *H. violacea* plants developed roots at 20°C, compared with 80% at 15°C and none at 10°C (Table 2). This suggests that the optimal temperature for development of roots from *H. violacea* cuttings is close to 20°C.

Generally auxin treatments with IBA, NAA, or other rooting accelerants are used when propagating by cuttings, to accelerate root formation. In this experiment, when treatments with 16, 40, and 80 ppm IBA were applied onto the cuttings beforehand, a high rate of root development was obtained (Table 3). In *Dorycnium*, 25 $mg \cdot L^{-1}$ and 200 $mg \cdot L^{-1}$ IBA promoted root development (Alegre et al., 1998). In olives, when cuttings were treated with 0.8% IBA, this promoted a high level of root development (Wiesman and Lavee, 1995). In plum trees, high rates of root development were obtained from treatment with 5000 ppm IBA for 1-5 s, or 50 ppm for 18 min. (Nahlawi and Howard, 1972).

In this experiment we found that maximum root production in *H. violacea* cuttings was possible with a comparatively low concentration (16 ppm) of IBA in Oxyberon solution. However, since the suitability of IBA treatment varies depending upon both the type of plant (Yamazaki et al., 1982), and on the treatment duration, more detailed investigations on IBA concentration and duration need to be carried out in order to find a simpler treatment method.

Usually cuttings are shielded from light to prevent rises in temperature, but strong light conditions have been shown to promote photosynthesis in the cuttings, and to promote the movement of auxins to the base of the cuttings (Gemma, 1997). In *Forsythia* when green cuttings were held at photophases ranging from 16-24 h, root development was promoted more strongly than when cuttings were kept in a 12-h photophase (Hata et al., 2009).

In this experiment, there was greater root development under a 16-hour photophase than under photophases of 10 and 12 hours. It was thought that the relatively weak light intensity (10 $\mu mol \cdot m^{-2} \cdot s^{-1}$, meant that a longer photophase was required to enable migration of auxins to the base of the cuttings.

Uda et al. (1994) added the non-ionic surfactant polyoxyethylene lauryl ether to silver thiosulfate solution (STS) for pre-treatment of spray carnations, and found that the retention period was far longer than when STS was used alone. Funakoshi (1988) also found that the addition of neutral detergents enhanced the retention effect of STS when applied to *Gypsophila* and *Bubarujia*. In this experiment, when 0.05% of the surfactant Tween 20 was mixed with 16 ppm IBA Oxyberon solution, high rooting rates were obtained (Table 4). Surfactants promote water absorption in cut flowers and improve water balance (Durkin, 1980; van Doorn et al., 1993). The Tween 20 surfactant used in this experiment is a type of polyoxyethylene lauryl ethers, effective in promoting water absorption in cut flowers. When perennial sweet pea cut flowers were treated with 0.05 mM STS in 10% sucrose with 0.05% Tween 20 for 2 h, the shelf life was extended (Koike and Imanishi, 2009).

This experiment has also shown that root development increases when a surfactant is mixed with rooting accelerant, and it is thought that this is because it promotes water absorption of cuttings.

The above results have demonstrated that high rooting rates can be obtained in *H. violacea* cuttings if they are soaked after cutting for 60 min. in 16 ppm IBA in

Oxyberon with 0.05% surfactant added, and cultured in rock wool at 20°C and LD 16:8 conditions.

Literature Cited

Algre, J., Toledo, J.L., Martinez, A., Mora O. and De Andres, E.F. 1998. Rooting ability of *Dorycnium* spp. under different conditions. Scientia Hort. 76:123-129.

Australian Native Plants Society. 2012. *Hardenbergia violacea*. <http://anpsa.org.au/h-viol.html>, Accessed 6 May 2012.

Durkin, D. 1980. Factors effecting hydration of cut flowers. Acta Hort. 113:109-117.

Fujihara, K. 1984. Cutting propagation. p.57-68. In: T. Tsukamoto (ed.), Encyclopedia of floriculture. Yokendo, Tokyo.

Fujihara, K. 1984. Propagation from cuttings. p.57-58. In: T. Tsukamoto (ed.), Genshoku Encyclopedia of Floriculture. Yokendo, Tokyo. [in Japanese].

Fujii, T. 1968. Principle and methods of cuttings. p.29-114. In: T. Fujii (ed.), Vegetative Propagation of Horticultural Plants. Seibundo-shinkosha, Tokyo.

Funakoshi, K. 1988. Effect of synthetic detergents on vase life of cut flowers. Abstr. Japan. Soc. Hort. Sci. Spring Meet.: p.446-447.

Funakoshi, K. 1988. Effects of neutral detergents on the vase life of different cut flowers. Horticulture Abstracts 63 (Spring), p.446-447. [in Japanese].

Gemma, H. 1997. Rooted cutting production. p.36-46. H. Imanishi and M. Tanaka (eds.), In Horticultural Seeds and Seedlings Production. Asakura-shoten, Tokyo.

Genma, H. 1997. Propagation by cuttings. p.36-46. In: H. Imanishi and M. Tanaka. (eds.), Horticultural Propagation Science. Asakura Shoten, Tokyo. [in Japanese].

Hata, N., Okazawa, A., Orimoto, K., Ono, E., Satake, H. and Kobayashi, A. 2009. Effects of IBA treatment, fertilization, photoperiod and light intensity on rooting of softwood cuttings of *Forsythia suspense*. J. SHITA. 21:15-23. (in Japanese with English summary).

Imanishi, H. 2000. Floriculture. Kawashima-shoten. Tokyo. [in Japanese].

Koike, Y. and Imanishi, H. 2009. Effects of silver thiosulfate complex (STS), sucrose, surfactant and their combination on the vase life of cut flower of *Lathyrus latifolius* L. Acta Hort. 813:679-684.

Nahlawi, N. and Howard, B.H. 1972. Rooting response of plum hardwood cuttings to IBA in relation to treatment duration and cutting moisture content. J. Hort. Sci. 47:301-307.

Rein, W.H., Wright, R.D. and Seiler, R. 1991. Propagation medium moisture level influences adventitious rooting of woody stem cuttings. J. Amer. Soc. Hort. Sci. 116:632-636.

Tilt, K.M. and Bilderback, T.E. 1987. Physical properties of propagation media and their effects on rooting of three woody ornamentals. HortSci. 22:245-247.

Uda, A., Koyama, Y., Fukushima, K. and Ikeda, Y. 1994. Effects of silver thiosulfate (STS) with or without a surfactant on the vase life of spray carnations (*Dianthus caryophyllus*). J. Japan. Soc. Hort. Sci. 63:645-652. (in Japanese with English summary).

Wiesman, Z. and Lavee, S. 1995. Enhancement of IBA stimulatory effect on rooting of olive cultivar stem cuttings. Scientia Hort. 62:189-198.

Yamazaki, K., Okabe, M. and Takahashi, E. 1982. Studies on the efficient propagation method for ornamental trees and shrubs by closing the rooting bed with polyethylene film. Effects of collecting date of cuttings and IBA treatment on rooting, and searching for the suitable woody species for this system. Bull. Kanagawa. Hort. Expt. Stat. 29:80-90. (in Japanese with English summary).