Responses to Propagation Substrate and Rooting Hormone Products to Facilitate Asexual Propagation of *Silene chalcedonica* and *Silene coronaria*[1]

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Abstract

Many ornamentals are commercially propagated by cuttings, which is an easy and cost-effective propagation method. *Silene* L. is a promising genus for extensive use as a landscape ornamental due to its drought tolerance, profuse flowering, and perennial characteristic. *Silene* is readily cross pollinated, so to ensure genetic purity for breeding and cultivar release, an asexual cutting propagation protocol needs to be established. Adventitious rooting of cuttings of *Silene chalcedonica* (L.) E.H.L. Krause and *Silene coronaria* (L.) Clairv. were evaluated using two different propagation substrates and three different root-promoting hormone products. Rooting percentage of *S. chalcedonica* cuttings was improved by Dip 'N' Grow treatment (1,000 $mg \cdot L^{-1}$), which contains 1% indole-3-butyric acid and 0.5% 1-napthaleneacetic acid, and Hormex Rooting Powder No. 1 (1,000 $mg \cdot L^{-1}$), which contains 0.10% indole-3-butyric acid; however, 89% of *S. coronaria* cuttings rooted readily in vermiculite without an auxin treatment. Therefore, rooting success of stem cuttings was highly species related. Because of the relatively low rooting percentage seen in vermiculite (46%) and in perlite (63%) using Dip 'N Grow auxin treatment for *S. chalcedonica*, an effective stem cutting propagation method still needs to be explored for this species.

Index words: *Lychnis*, Maltese cross, rose campion, cuttings, vermiculite, perlite, hormone, rooting.

Species used in this study: Maltese cross [*Silene chalcedonica* (L.) E.H.L. Krause] and rose campion [*Silene coronaria* (L.) Clairv].

Chemicals used in this study: rooting hormone (Hormex Rooting Powder No. 1, indole-3-butyric acid, 0.10%); rooting hormone (Dip 'N Grow liquid rooting concentrate, indole-3-butyric acid, 1% and 1-napthaleneacetic acid, 0.5%); rooting hormone (Hortus IBA Water Soluble Salts, indole-3-butyric acid 20.0%). Note: All commercial products used in this research are not for advertisement, and data related to any commercial products were solely generated for this publication.

Significance to the Horticulture Industry

Asexual propagation by stem cuttings is important in the horticultural industry for mass production of ornamentals quickly and inexpensively while retaining genetic purity. Maltese cross (*Silene chalcedonica* (L.) E.H.L. Krause) and rose campion (*Silene coronaria* (L.) Clairv.) are old fashion garden ornamentals that flower profusely and have good drought tolerance. Asexual stem cutting propagation methods were investigated for the two *Silene* L. species based on the rooting effects with regard to three commercial rooting hormones and two propagation substrates. Dip 'N Grow (indole-3-butyric acid, 1% and 1-napthaleneacetic acid, 0.5%) hormone treatment with vermiculite as the substrate, and Hormex (indole-3-butyric acid, 0.10%) with either vermiculite or perlite favored the rooting of Maltese cross. However, vermiculite without rooting hormone was recommended for rooting of rose campion.

Introduction

Silene L., commonly known as campion or catchfly, is a genus of 24 species in the carnation family Caryophyllaceae Juss. (GRIN 2010). *Silene* species are native to temperate regions in the Northern Hemisphere (Popp 2008). Most species in this genus are of ornamental valuable because of the showy, bright colored flowers, which can be either magenta, red, orange, crimson, pink, or white. *Silene chalcedonica*, also known as Maltese cross or Jerusalem cross, is a perennial ornamental flower that has been grown in North American since colonial times. It can be grown in USDA Zones 3 to 10. *Silene coronaria*, with the common name of rose campion, is a short-lived perennial plant generally grown as a biennial or annual ornamental flower in USDA Zones 4 to 8. Vegetative propagation, including adventitious rooting of stem cuttings, is essential and widely used for producing ornamental plants. There are many factors that affect rooting, such as accurate control of moisture, temperature, light, hormone formulation and concentration, media, and stock plant quality. For example, light intensity (Park et al. 2011) and plug cell size (Park et al. 2010) affect rooting of rose (*Rosa hybrida* L.) cuttings, and even the production of rose cut flowers.

No asexual propagation methods have been published (leaf or stem cutting) for campion species (*Silene spp.*), although procedures are continually investigated for its family member carnation (*Dianthus* spp.). Peat plus perlite at a volume ratio 70:30 and mist irrigation that maintained ambient relative humidity around 75 to 85% worked well for carnation cuttings (Garrido et al. 1996, 1998, 2002). Cold storage is a standard commercial procedure for stem cuttings of *Dianthus caryophyllus* L. (carnation) to promote roots (Holley and Baker 1990). Previous work (Garrido et al. 1996) demonstrated that cold storage and hormone treatment influenced rooting of three cultivars of carnation differently. The investigators hypothesized that different cultivars may have various endogenous auxin levels. Research on cold storage and fresh cuttings plus exogenous auxin application were carried out on another two carnation cultivars, and results supported the hypothesis (Garrido et al. 1998). Garrido et al. (2002) revealed that mature leaves attached on carnation

[1]Received for publication December 7, 2015; in revised form July 12, 2016.

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cuttings are essential to rooting since indole acetic acid (IAA) translocates from mature leaves to stems and can promote root development, while cuttings with only immature leaves need 24 additional days to allow the juvenile leaves to mature for root initiation. However, carnation develops roots readily. Therefore, the effect of exogenous rooting hormone on more recalcitrant species should be greater.

Plenty of cutting propagation techniques and commercial rooting products are available to facilitate better root of cuttings for floriculture crops (Dole and Gibson 2006). Indole-3-butyric acid (IBA) is the common active ingredient either formulated in water (potassium salt, K-IBA), alcohol (free acid of IBA), or talc depending on the chemical formulation and rooting requirements of the cuttings (Crawford 2005). These products are usually used on species whose stem cuttings are difficult to root, such as hardwood and semi-hardwood cuttings, and some herbs. A risk associated with exogenous auxin application is phytotoxicity (species, formulation, and concentration dependent) which can reduce rooting success.

As cultivars of *Silene* become more popular, a well-defined cutting procedure will need to be established to meet consumer demand. Chen et al. (2006) established tissue culture protocols for *Silene banksia* (Meerb.) Mabb. (synonym of *L. senno* Siebold & Zucc. and common name Ganpi in Japanese and Jian Chun Luo in Chinese). However, developing a protocol for stem or leaf cuttings would allow for more widespread propagation of improved ornamental cultivars. The purpose of this experiment was to establish a protocol for vegetatively propagating *S. chalcedonica* and *S. coronaria*.

Materials and Methods

Seeds of *S. chalcedonica* and *S. coronaria* bought from J. L. Hudson, Seedsman (La Honda, CA) were planted in March 2011 and May 2010, respectively, in the Oklahoma State University Horticultural Department Research Greenhouses, Stillwater under natural photoperiods. Temperature was set at 21/18.3 C (70/65 F) day/night with a photosynthetic photon flux density (PPFD) range of 600 to 1,200 $\mu mol \cdot m^{-2} \cdot s^{-1}$at 1200 HR. Plants were watered as needed with non-pressure compensated 0.2 cm diameter drip emitters (Chapin Watermatics Inc. Watertown, NY) until water ran through the pots. Plants were fertigated at each watering with 200 $mg \cdot L^{-1}$ 20-10-20 N-P-K (Jack's Professional® General Purpose acidic fertilizer, J.R. Peters Inc., Allentown, PA).

On June 28, 2011, stem cuttings of *S. chalcedonica* were collected. Terminal stem cuttings were 0.8 cm (0.3 in) in length and had four pairs of leaves. Cuttings were then trimmed below the top two pairs of leaves from the meristem. On August 9, 2011, cuttings of *S. coronaria* were collected. Terminal stem cuttings were 0.5 cm (1.3 in) in length with a single leaf and a small lengthwise part of stem tissue to ensure the stems included an axillary bud for vegetative growth after rooting. Stem cuttings of each species were either not treated with a hormone (untreated control) or treated immediately after harvesting with one of three root promoting hormones, and then inserted into a cell in a 30 by 50 cm (12 by 20 in) flat tray (T.O. Plastics, Clearwater, MN) with one of two rooting substrates. Each cutting received a five second dip in a hormone concentration of 1,000 $mg \cdot L^{-1}$ (ppm). Hormones used included Hormex No. 1 rooting powder (IBA in talc, indole-3-butyric acid, 0.10%, Brooker Chemical, Chatsworth, CA), Dip 'N Grow liquid rooting concentrate (IBA and NAA dissolved in alcohol, indole-3-butyric acid, 1% and 1-napthaleneacetic acid, 0.5%, (DIP 'N GROW, Inc., Clackamas, Oregon), and Hortus IBA water soluble salts (Hortus USA Corp., New York, NY, indole-3-butyric acid 20.0%). Two substrates with different air porosity were used for growing cuttings, including 100% perlite (Sun Gro Horticulture, Agawam, MA) and 100% vermiculite (Sun Gro Horticulture). For air porosity, perlite was rated as high category and vermiculite as medium, as the perlite tended to drain water more readily and was more porous than the vermiculite. Stem cuttings were placed randomly on a greenhouse bench under intermittent mist operating 8 seconds per minute for *S. chalcedonica*. Because a high humidity condition readily causes decay of stem cuttings in *S. coronaria*, a less humid condition by mist operating 15 seconds every 32 minutes was used for *S. coronaria*.

Data collected on *S. chalcedonica* cuttings included percent rooting, root number per rooted cutting, and root length for each rooted cutting. Percent rooting was defined as the number rooted cutting divided by the 15 cuttings for each replicate. For root numbers, because roots were too fine to be counted individually after drying, the median number of the estimated range was recorded. It was noted as follows: if root number was within the range 1 to 5, then it was recorded as 3; if root number was between 6 to 10, then it was 8; if root number was between 11 to 15, then it was recorded as 13. Data collected on *S. coronaria* included percent of rooted cuttings, root length, and root dry weight as an alternative of root number. An alternative observation, root weight was measured for *S. coronaria* because it had more root biomass compared to *S. chalcedonica*. Roots were cut from the stem and placed in coin envelopes then put into a Precision Scientific Oven (Jouan Inc., Winchester, VA) at 70 C (158 F) for 48 hours then weighed (Somasegaran et al. 1983).

For each species, the experimental design was a two by three factorial in a randomized complete block design. Each treatment was replicated six times and had 15 cuttings per replication for *S. chalcedonica* (90 cuttings per treatment total), and four replications with 15 cuttings per replication for *S. coronaria* (60 cuttings per treatment total). Data were analyzed by SAS/STAT® software (SAS/Stat procedures, Version 9.2; SAS Institute, Inc., Cary, NC) using generalized linear mixed models within the GLIMMIX procedure and mean separation using the Tukey post hoc test.

Results and Discussion

Both substrate and hormone treatments, and their interaction, significantly influenced root length in both species and influenced rooting percent in *S. coronaria*, whereas only hormone treatment significantly influenced rooting percent in *S. chalcedonica* (Tables 1 and 2). Similar to rooting percent, the hormone treatments significantly influenced root number in *S. chalcedonica* (Table 1). The interaction effect between substrate and hormone was significant for *S. coronaria* (Table 2).

Considering the effectiveness of substrates and hormone products tested on two species, vermiculite, which can hold more water, favored root development for stem cuttings of *S. chalcedonica* when combined with either Dip 'N Grow or Hormex treatments (Tables 3 and 4). Compared with the control, Dip 'N Grow application increased rooting percent of *S. chalcedonica* from 22.2 to 45.6% grown in vermiculite and from 20.0 to 63.3% in perlite, respectively (data not

Table 1. **Analysis of variance for effects of substrates, hormone products, and their interactions on root growth of *Silene chalcedonica* cuttings.**

		Rooting percentage		Root number		Root length	
Effect	**DF**	**F value**	**Pr>F**	**F value**	**Pr>F**	**F value**	**Pr>F**
Substrate (S)	1	0.28	0.5982	0.26	0.6103	29.53	<0.0001
Hormone (H)	3	17.94	<0.0001	24.84	<0.0001	10.31	<0.0001
S × H	3	1.83	0.1581	0.25	0.8622	3.59	0.0221

Table 2. **Analysis of variance for effects of substrates, hormone products, and their interactions on root growth of *Silene coronaria* cuttings.**

		Rooting percentage		Root weight		Root length	
Effect	**DF**	**F value**	**Pr>F**	**F value**	**Pr>F**	**F value**	**Pr>F**
Substrate (S)	1	11.65	0.0026	1.62	0.2188	8.74	0.0072
Hormone (H)	3	7.79	0.0011	2.53	0.0877	7.01	0.0017
S × H	3	38.95	<0.0001	7.10	0.0022	34.13	<0.0001

shown). The greatest number of stem cuttings (88.6%) of *S. coronaria* rooted in vermiculite occurred without hormone; all hormone products in this study interfered negatively with rooting (Table 5). Root growth f *S. coronaria* in perlite was poor and Dip 'N Grow and Hortus treatments improved rooting percentage of *S. coronaria* in this substrate significantly to 75.1 and 80.95%, respectively, compared to the control of 1.7% (Table 5).

Variation among species in terms of rooting success has been widely observed in ornamental flowers (Griffith 1998, Hartmann et al. 2002, Blythe et al. 2004). Root-inducing auxin products for cutting propagation commonly contain indole-3-butyric acid (IBA), 1-naphthaleneacetic acid (NAA), or a combination of the two chemicals (Laubscher et al. 2008, Baig et al. 2011, Sharma et al. 2006). The present study revealed contrasting responses of two species to auxin treatments in the genus of *Silene chalcedonica* cuttings rooted 45.6% in vermiculite and 63.3% in perlite using the Dip 'N Grow hormone treatment, while 88.6% of *S. coronaria* cuttings rooted readily in vermiculite without an auxin treatment.

In addition to the species difference, benefits of auxin treatments depends on auxin concentration, condition of the cutting plants, application methods (such as the quick-dip method and foliar spray method), substrates and other factors (Ari 2016, Anches and Maria 2012, Blythe et al. 2004, Laubscher and Ndakidemi 2008, Lazaj et al. 2015, Nair et al. 2008, Smitha and Umesha 2012, Sevik and Guney 2013). Also, cold storage was observed to promote rooting of cuttings in the *Dianthus* family (Holley and Baker 1990). In this study, stem cuttings were collected from approximately 4-month old plants for *S. chalcedonica*, but 15 month old plants were cut for *S. coronaria*. The growing length of stem cutting for *S. coronaria* was 16 days longer than for *S. chalcedonica*. Further studies may investigate these details for improving rooting of stem cuttings for *S. chalcedonica*.

S. coronaria is an obligate vernalization species for reproductive growth (Chouard 1960). Zoberi et al. (2003) reported that adding light to extend daytime caused *Achillea filipendulina* Lam. cuttings to flower year round without vernalization no matter when the cuttings were taken from induced plants or non-induced plants. Establishing an asexual propagation

Table 3. **Percent rooting and root number of *Silene chalcedonica* cuttings responding to different root promoting auxin products at 1,000 mg·L^{-1} (ppm), averaged over rooting medium.**

Product	**Hormone treatment**	**Rooting percentage (%)**	**Mean root number**
Dip 'N Grow	1% IBA[z] and 0.5% NAA[y]	54.93a[x]	4.044a[x]
Hormex	0.10% IBA	52.81a	3.272ab
Hortus	20.0% IBA	46.04a	2.611b
Control	None	19.40b	0.883c

[z]IBA, indole-3-butyric acid.

[y]NAA, 1-Napthaleneacetic acid.

[x]Different letters in the column indicate differences are significant at $P \leq 0.05$ based on the Tukey post hoc test.

Table 4. **Interaction of rooting substrate and root promoting auxin product at 1,000 mg·L^{-1} (ppm) on root length and root weight of *Silene chalcedonica* cuttings.**

Substrate	**Hormone treatment**	**Mean root length (mm)**	**Total root weight (g)**
Vermiculite	Control	19.15bc[x]	114.12cd[x]
Vermiculite	Dip 'N Grow (1% IBA[z], 0.5% NAA[y])	32.44a	352.64a
Vermiculite	Hormex (0.10% IBA)	32.26a	287.59ab
Vermiculite	Hortus (20.0% IBA)	19.34bc	125.00cd
Perlite	Control	7.60c	31.55d
Perlite	Dip 'N Grow (1% IBA[z], 0.5% NAA[y])	17.44bc	148.14bcd
Perlite	Hormex (0.10% IBA)	19.86b	190.52bc
Perlite	Hortus (20.0% IBA)	19.42bc	118.34cd

[z]IBA, indole-3-butyric acid.

[y]NAA, 1-Napthaleneacetic acid.

[x]Different letters in the column indicate differences are significant at $P \leq 0.05$, n = 90 based on the Tukey post hoc test.

Table 5. **Interaction of rooting substrate and root-promoting auxin product at 1,000 mg·L^{-1} (ppm) on percent rooting, root length, and root weight of *Silene coronaria* cuttings.**

Substrate	Product	Rooting percentage (%)	Root length (mm)	Root weight (g)
Vermiculite	Control	88.56a[x]	31.67a[x]	0.0405ab[x]
Vermiculite	Dip 'N Grow (1% IBA[z], 0.5% NAA[y])	48.32bc	7.40bcd	0.0254ab
Vermiculite	Hormex (0.10% IBA)	56.90b	9.32bcd	0.0387ab
Vermiculite	Hortus (20.0% IBA)	18.03cd	1.25cd	0.0620a
Perlite	Control	1.69d	0.12d	0.0260ab
Perlite	Dip 'N Grow (1% IBA[z], 0.5% NAA[y])	75.05ab	12.08bc	0.0667a
Perlite	Hormex (0.10% IBA)	0.43d	0.13d	0.0122b
Perlite	Hortus (20.0% IBA)	80.87ab	16.75b	0.0296ab

[z]IBA, indole-3-butyric acid.

[y]NAA, 1-Napthaleneacetic acid.

[x]Different letters in the column indicate differences are significant at $P \leq 0.05$, n = 60 based on the Tukey post hoc test.

method for *S. coronaria* might also be an effective way to transfer vernalization or substituting for the vernalization requirement through manipulating environmental factors of the cuttings.

In summary, contrasting responses of two *Silene* species to auxin treatments were observed. While rooting percent of *S. chalcedonica* was improved by 1,000 mg·L^{-1} Dip 'N Grow and grown in vermiculite, 88.6% of *S. coronaria* rooted readily in vermiculite without an auxin treatment. Therefore, stem cutting performance was highly species related. Regarding the relatively low rooting percent for *S. chalcedonica*, an effective stem cutting propagation method still needs to be developed.

Literature Cited

Anches, C. and B. Maria. 2012. Results in *Ficus australis* rooting on different substrata and with different rooting enhancers with effect on cutting rooting in the fall. J. Hort. For. Biotech. 16:105–109.

Ari, E. 2016. Effects of different substrates and IBA concentrations on adventitious rooting of native *Vitex agnus-castus* L. cuttings. Acta Scientiarum Polonorum Hortorum Cultus 15(2):27–41.

Baig, M.M.Q., I.A. Hafiz, A. Hussain, T. Ahmad, and N.A. Abbasi. 2011. An efficient protocol for in vitro propagation of *Rosa grussanteplitz* and *Rosa centifolia*. African J. Biotech. 10:4564–4573.

Blythe, E.K., J.L. Sibley, J.M. Ruter, and K.M. Tilt. 2004. Cutting propagation of foliage crops using a foliar application of auxin. Sci. Hort-Amsterdam 103:31–37.

Chen, L.P., Y.J. Wang, C.X. Xu, M.S. Zhao, and J.G. Wu. 2006. In vitro propagation of *Lychnis senno* Siebold et Zucc., a rare plant with potential ornamental value. Sci. Hort-Amsterdam 107:183–186.

Chouard, P. 1960. Vernalization and its relations to dormancy. Ann. Rev. Plant Physiol. 11:191–238.

Crawford, M. 2005. Methods and techniques to improve root initiation of cuttings. Intl. Plant Propagators Soc., Combined Proceedings 55:581–585.

Dole, J.M. and J.L. Gibson. 2006. Cutting Propagation: A Guide to Propagating and Producing Floriculture Crops. Ball Publishing. 400 pp.

Garrido, G., E.A. Cano, M.B. Arnao, M. Acosta, and J. Sanchez-Bravo. 1996. Influence of cold storage period and auxin treatment on the subsequent rooting of carnation cuttings. Sci. Hort-Amsterdam 65:73–84.

Garrido, G., E.A. Cano, M. Acosta, and J. Sanchez-Bravo. 1998. Formation and growth of roots in carnation cuttings: Influence of cold storage period and auxin treatment. Sci. Hort-Amsterdam 74:219–231.

Garrido, G., J.R. Guerrero, E.A. Cano, M. Acosta, and J. Sanchez-Bravo. 2002. Origin and basipetal transport of the IAA responsible for rooting of carnation cuttings. Physiol. Plant. 114:303–312.

Griffith Jr., L.P., 1998. Tropical Foliage Plants: A Grower's Guide, 2nd ed. Ball Publishing, Batavia, IL. 400 pp.

Hartmann, H.T., D.E. Kester, F.T. Davies Jr., R.L. Geneve. 2002. Hartmann and Kester's Plant Propagation: Principles and Practices, 7th ed. Prentice-Hall, Upper Saddle River, NJ. 880 pp.

Holley, W.H. and R. Baker. 1990. Carnation Production II. Kendall Hunt Publishing, Dubuque, USA. 156 pp.

Laubscher, C.P. and P.A. Ndakidemi. 2008. Rooting success using IBA auxin on endangered *Leucadendron laxum* (Proteaceae) in different rooting mediums. African J. Biotech. 7:3437–3442.

Lazaj, A., P. Rama, and H. Vrapi. 2015. The interaction with season collection of cuttings, Indol Butyric Acid (IBA) and juvenility factors on root induction in *Olea europaea* L. (Cultivar 'Kalinjot'). Intl. Refereed J. Eng. Sci. 4:32–38.

Nair, A., D. Zhang, J. Smagula, and D. Hu. 2008. Rooting and overwintering stem cuttings of *Stewartia pseudocamellia* Maxim. relevant to hormone, media, and temperature. Hort. Sci. 43:2124–2128.

Park, Y.G. and B.R. Jeong. 2010. Effect of plug cell size seed in propagation on the growth and yield of stenting-propagated cut roses. Hort. Environ. Biotech. 51:249–252.

Park, S.M., E.J. Won, and B.R. Jeong. 2011. Effects of node position, number of leaflets left, and light intensity during cutting propagation on rooting and subsequent growth of domestic roses. Hort. Environ. Biotech. 52:339–343.

Popp, M. 2008. New combinations in *Lychnis* (*Caryophyllaceae*) from Africa. Novon: A J. Botanic Nomenclature 18:99–100.

Sevik, H. and K. Guney. 2013. Effects of IAA, IBA, NAA, and GA_3 on rooting and morphological features of *Melissa officinalis* L. stem cuttings. The Scientific World Journal, 2013. 5 pp.

Sharma, J., G.W. Knox, and M.L. Ishida. 2006. Adventitious rooting of stem cuttings of yellow-flowered *Magnolia* cultivars is influenced by time after budbreak and indole-3-butyric acid. Hort. Sci. 41:202–206.

Smitha, G.R. and K. Umesha. 2012. Vegetative propagation of Stevia [*Stevia rebaudiana* (Bertoni) Hemsl.] through stem cuttings. J. Trop. Agric. 50(1–2):72–75.

Somasegaran, P., R. Woolfenden, and J. Halliday. 1983. Suitability of oven-dried root nodules for *Rhizobium* strain identification by immunofluorescence and agglutination. J. Appl. Bact. 55:253–261.

Zoberi G., S. Carmi, D. Evenor, E. Shlomo, and M. Reuveni. 2003. Rooted cuttings of *Achillea filipendulina* 'Parker' will flower without vernalization. J. Hort. Sci. Biotech. 78:100–103.