HYBRIDIZATION, GENETIC MANIPULATION, AND ASEXUAL PROPAGATION OF LYCHNIS SPECIES

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LYCHNIS SPECIES

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CHAPTER I

INTRODUCTION

Lychnis L., commonly known as campion or catchfly, is a genus of 24 species in the carnation family Caryophyllaceae Juss. (GRIN, 2010). *Lychnis* species are native to temperate regions in the Northern Hemisphere (Popp et al., 2008). Most species in this genus are of ornamental value, because of the showy, bright colored flowers which can be either magenta, red, orange, crimson, pink, or white. Species of *Lychnis* are most commonly cultivated. However, a number of cultivars selected from *Lychnis chalcedonica* L., *Lychnis xhaageana* Lemoine, *Lychnis flos-cuculi* L., *Lycnhis flos-jovis* (L.) Desr., *Lycnhis coronaria* (L.) Desr., and *Lycnhis viscaria* L. are also available (Table A.1). Most *Lychnis* species are perennial, ornamental herbs that flower continuously for 2 to 3 months from May to July. Some *Lychnis* species are annuals in cooler climates, but no reports described the winter hardiness levels for *Lychnis*. According to some ornamental seed induatries, *L. flos-cuculi* can survive in hardiness zones 3 or 4, and *L. coronaria* and *L. flos-jovis* can survive in zones 5-8. We observed *L. coronaria* and *L. flos-cuculi* to be hardy in our research field (USDA hardiness zone 6B) in spring 2011after being transplanted outside in May 2010. According to our observation in the greenhouses, *L. coronaria*, *L. coronaria* var. *alba*, *L. flos-jovis*, *L. wilfordii* (Regel) Maxim., *L. xhaageana, L. viscaria*, and *L. alpina* L. have great drought-stress tolerance, but favor well drained soil. *Lychnis chalcedonica* can endure drought slightly better than *L. chalcedonica* var. *alba*. *Lychnis flos-cuculi* needs lots of water initially, but once its tufted leaves cover the ground surface, its drought tolerance is better than *L. chalcedonica*.

There are 86 genera accepted in the family Caryophyllaceae Juss. (GRIN, 2003). The boundary between the genera *Lychnis* and *Silene* L. are often disputed in the field of taxonomy and botany (Desfeux and Lejeune, 1996; Kruckeberg, 1962). The debate has existed for over a half century as taxonomists are unsure into which genus (*Lychnis, Silene,* or the old genus *Melandrium* Röhl) to place species (Desfeux and Lejeune, 1996; Kruckeberg, 1962). The genera *Lychnis* and *Silene* have been merged in cladistic theory (Desfeux and Lejeune, 1996; Negrutiu et al., 2001), although all species in the genus *Lychnis* now share two different scientific names which belong to genera *Lychnis* and *Silene,* respectively (GRIN, 2010). Some reseachers prefer to maintain the old classification. Širokỳ et al. (2001) adopted a broad *Silene* genus comprising about 700 species including *Lychnis*, but treated *Lychnis* as an independent group among 44 sections in the genus *Silene*. Phylogenic relation could tell the evolutionary relationship among species in *Lychnis* or species between genera *Lychnis* and *Silene*. For example, molecular phylogeny indicates that *L. coronaria*, *L. flos-jovis* and *S. nutants* L. are all related (Fior et al., 2006). *Lychnis xwalkeri* is a putative hybrid between *L. coronaria* and *L. flos-jovis*. *Lychnis flos-cuculi* and *L. coronaria* are closely related (Desfeux and Lejeune, 1996), which is of great interest in hybridization since the two species are quite different in natural characteristics of morphology, physiology, as well as habitat, yet no hybrids exist. *Lychnis chalcedonica*, *L. flos-cuculi* and *L. flos-jovis* are close to each other in phylogenetic analysis (Erixon and Oxelman, 2008), while *Silene armeria* L. is pretty close to *L. viscaria* in phylogenetic analysis (Desfeux and Lejeune., 1996), yet these species have no direct evidence showing their cross-compatibility so far.

With the exception of hybrid *L. xwalkeri* mentioned above, the only other horticultural hybrid known is *L. xarkwrightii*, which is a cross between *L. chalcedonica* and *L.* x*haageana*. Distinction between the traditional genus *Lychnis* and *Silene* in morphology is that *Lychnis* usually has five styles and an entire capsule, while *Silene* has three styles and a split capsule (Desfeux and Lejeune, 1996).

Lychnis exhibits high diversity in morphology and physiology. For example, *L. xhaageana* has dark green elliptical leaves on upright branched stems bearing red or orange flowers with a diameter of 3-4 cm, while *L. chalcedonica* has green to dark green elliptical leaves on long, flexual branched stems with five small petals and clustered red flowers. *Lychnis flos-cuculi* has longer oblanceolate and slightly wrinkled leaves. *Lychnis viscaria* and *L. alpina* have dark green leaves either ensiform or linear, and all of them have tufted leaves near the ground with flowers on top of fertile stems high above the leaves. *Lychnis coronaria* and *L. coronaria* var. *alba*, also known as rose campion, are old fashion garden ornamentals with silver-gray colored oblanceolate or oval leaves, which form a tuft in the vegetative growth stage. Rose campion has opposite leaves on aerial stems, and has pink, purple, or white flowers of at least 2.5 cm in diameter.

Based on our observation, majority of *Lychnis* species are hermaphrodite and prefer cross-pollination, which is aided by protandry, though self-pollination does occur and quite often in a few species. Research done concerning selfing and outcrossing in *Lychnis* species has focused on *L. flos-cuculi*, *L. viscaria* and *L. alpina* since they represent ecological plants. Most *Lychnis* species are cross pollinated by insects. Floral fragrance and its composition play important roles for attraction and determination of pollinators (Andersson et al., 2002; Proctor et al., 1996; Miyake et al., 1998). Moths were observed to be visitors of *L. flos-cuculi* and cultivars as well as *L. chalcedonica* when in the juvenile growth stage. It is speculated that there are some special chemical components in the leaves rather than floral scent to attract moths, since moths obviously favored the species *L. flos-cuculi* starting in the vegetative growth stage in our research population. Insect visitors for *Lychnis* and *Silene* species include bees, moths, butterflies, flies, and mosquitoes (Brantjes and Leemans, 1976; Ellis and Ellis-Adam, 1993; Jürgens et al., 1996, 2002; Van Rossum and Triest, 2010). Aphids were observed to be important *Lychnis* pollinators especially for *L. chalcedonica*, *L. chalcedonica* var. *alba*, and *L. flos-cuculi*, while ants were observed facilitating pollination for *L. flos-cuculi* in our greenhouses. Those *Lychnis* species with

relative large flowers (*L. xhaageana*, *L. cognate*, *L. vesuvius*), self-pollinate less in the greenhouse, while *L. chalcedonica* and *L. chalcedonica* var. *alba* have a high selfing rate. *Lychnis wilfordii* has a very low selfing rate although its individual flowers are nearly the same size as *L. chalcedonica*.

OBJECTIVE

This research is divided into three mutually related subjects including hybridization, genetic manipulation, and asexual propagation. The objectives are to 1) acquire novel and adaptive plants for release or for future breeding with the hypothesis that hybrids could be generated through intraspecific, interspecific, and intergeneric hybridization, 2) obtain desirable mutants through mutagen treatments, and evaluate the effects of caffeine as a chemical mutagen. The hypothesis for genetic manipulation is treating seeds with mutagens could lead to novel mutants among selected *Lychnis* species, and 3) attempt to establish effective asexual propagation procedures for selected *Lychnis* species with the hypothesis that certain media and hormone combinations would promote better rooting in *Lychnis* stem and leaf cuttings.

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CHAPTER II

HYBRIDIZATION BREEDING

INTRODUCTION

Hybridization has been used to test the phylogeny relationship of *Lychnis* L. and its related genus *Silene* L. (Kruckeberg, 1962; Wilson et al., 1995). Yet, the ornamental value of interspecific hybrids among *Lychnis* or intergeneric hybrids between genus *Lychnis* and *Silene* has not been reported. The main goal of this research was to generate novel hybrids among *Lychnis* species, and between the genera *Lychnis* and *Silene*. Selection for natural mutants among *Lychnis* species was also conducted. *Lychnis* is a genus in the carnation family, Caryophyllaceaee Juss. During hybridization and selection, a new question about breeding double flowers in *Lychnis* arose, so an attempt to initiate double flower breeding was attempted among natural *Lychnis* mutants with more petals, which were found in this experimental population. Also, crossings with mutants obtained from mutagenetic treatment in this project were tried.

From an ornamental perspective, species within *Lychnis* and *Silene* can be divided into two groups. One is a bigger flower size group including most of the *Lychnis* species collected in this research. The other one is a smaller flower group, which is represented by *L. flos-cuculi* L., *L. chalcedonica* L. and all *Silene* species collected for this research. An objective of this research was to produce hybrids between species with bigger flowers with species having smaller flowers. The majority of *Lychnis* and *Silene* species have identical somatic chromosome numbers (2n=2x =24) (Negrutiu et al., 2001, and references therein). However, flow cytometric analysis indicated *S. pendula* L. has a smaller genome size, and *L. chalcedonica* has a larger genome size (Negrutiu

et al., 2001). The two species are important representatives for the smaller group of flowers among our collection.

LITERATURE REVIEW

Most horticultural cultivars in the genus *Lychnis* were obtained from intraspecific breeding (Godo et al., 2009; Mayol et al., 2006). There are few interspecific hybrids produced with special taxonomical, ecological, or genetic research purposes other than for ornamental merit.

Research on *Lychnis* is extensive in the ecology field. Hauser and Loeschcke (1996) tested if drought stress could cause inbreeding depressing in *L. flos-cuculi*. *Lychnis viscaria* L. has been a research focus with respect to genetic diversity and fitness components in natural populations in its habitats as a conservation species (Wilson et al., 1995; Lammi et al., 1999). Mustajärvi et al., (2005) studied the effects of population mating, history, and nutrition on inbreeding depression in *L. viscaria*. There are other limited hybrids produced with special ecological research purposes, but no evidence has suggested their ornamental value. An example is the hybrids that resulted from crosses between *L. viscaria* and *L. alpine*, as the hybrids are sterile and suffer higher developmental instability (Böcher, 1977; Siikamäki, 1999).

Interspecific or intergeneric hybridization in *Lychnis* is mainly used in taxonomical studies, as Kruckeberg (1962) conducted intergeneric crossing among *Lychnis*, *Melandrium* Röhl, and *Silene* to assist in determination of a clear-cut boundary among the genera. Wilson et al. (1995) suspected a hybrid between *L. viscaria* L. (=*Viscaria vulgaris* Bernh.) and *S. nutants* L., known as 'Vislene Hybrida' existed as reported in previous research. Phylogeny results from Desfeux and Lejeune (1996) also show a great genetic distance between *L. viscaria* and *S. nutants* L. Kruckeberg (1962) reported crossing *L. drummondii* (Hook.) S. Wars. with several *Silene* species, and nearly all combinations set seeds and produced F_1 hybrids (Table A. 2).

Široký et al. (2001, and references therein) reported that except for two diploid species possessing chromosome number 2n=20 and some tetraploids, all species in *Silene* have genome 2n=2x=24, which is also the case for the majority of *Lychnis* species (Table A.3). Flow cytometric analysis indicated *S. vulgaris* (Moench) Garcke and *S. pendula* L. have a small genome size, but dioecious white campion (*S. latifolia* Poir.) and hermaphrodite *L. chalcedonica* have large genome size values (Negrutiu et al., 2001), which may pose challenges for interspecific and intergeneric crossing for ornamental interests among *Lychnis* species or between *Lychnis* and *Silene*.

MATERIALS AND METHODS

Seven *Lychnis* species, 12 varieties or cultivars, five *Silene* species, and two chemical mutagen treated *Lychnis* species were used in the hybridizations (Table 2.1). Five crosses including reciprocal crosses were attempted for each combination without emasculation, yet the actual crossing numbers were restricted to flower availability. Fertilized capsules were collected as the color changed from green to brown. All hybridizations and selfings were conducted during May to October, 2010.

Seeds from individual capsules were sown in Sun Gro Horticulture Metro-mix 702 (Seba Beach, Alberta, Canada) media placed in round pots (diameter 15 cm) starting July 2010. Beginning in July, 2010, the number of crosses, number of successful crosses, as well as F_0 seed number, morphology, and germination rates were recorded. Seed morphology was scored as either developed with a regular shape (DR), developed with an irregular shape (DI), developed yet flat (DF), underveloped with an irregular shape (UI), or undeveloped with a regular shape (UR). Phenotypic traits including leaf color, flower color and size, and F1 seed set for selected hybrids were recorded starting in May 2011. Hybrids were grown in the Oklahoma State University Horticulture Research Greenhouses, Stillwater. Flower and leaf color were recorded using The

Royal Horticultural Society Color Charts. Hybrid flower morphology data were analyzed by SAS/STAT® software using ANOVA procedure.

RESULTS

Over 1,200 crossing combinations among and within cultivars and species of *Lychnis* L. and *Silene* L., including reciprocal crosses, were made (Table B.1, B.2 and hybridization attempts between *Lychnis* and *Dianthus*, data not shown). Seeds from selfing were collected for purpose of exotic phenotype selection and doubled flower traits and were kept for future breeding. Self-pollination data were excluded from the results.

Regular Hybridization

Combinations listed in Table B. 1 are regular hybridizations to create novel hybrids, and contributed to the majority of the crossing research. There were 78 combinations out of 177 (Table B.1) that produced hybrid seeds yet without germination (Table 2.2), and 28 combinations out of 177 that produced hybrid seeds with germination success under greenhouse conditions (Table 2.3). Hence, a total of 106 crossing combinations had seeds set despite germination issues. In Table 2.2, there were no F_0 seeds that germinated, and the sum of developed yet irregular (DI), developed yet flat (DF), undeveloped and irregular (UI), and undeveloped yet regular (UR) seeds count up to 90.6% of total hybrid seeds, whereas, in Table 2.3, for the hybridization with germinated seeds, F_0 seeds combinations of DI, DF, UI, and UR seeds were 21.8% of the total seeds.

Eleven (Code No.1, 2, 3, 4, 17, 18, 37, 38, 51, 52 & 66) out of 28 hybrids are derived from intra-specific hybridizations of *L. chalcedonica* (Table 2.3). Seed germination rates were high for these hybrids. The original record shows that cross combinations (Code No. 1, 17, 18, 51& 52) which had very low germination rates were all planted from mid-July to mid-August, 2010, and the propagated seeds gave a very low germinate rate until the greenhouse temperatures went

down. The high temperatures in the summer adversely affected germination rate of the species *L. chalcedonica*. These intraspecific crossings did not give unusual results. The F1 plants were all the same red flower color as *L. chalcedonica* regardless of the parent flower color including red (*L. chalcedonica*), white (*L. chalcedonica* var. *alba*, *L. chalcedonica* 'White', and *L. chalcedonica* 'Rauhreif'), or dusty pink (*L. chalcedonica* 'Carnea') .

Fifteen of the hybrids had phenotypes that obviously differ from their parents including flower color (Table 2.3 and 2.4), while some hybrids had traits intermediate of both their parents. For example, stem color, stem hardness, and leaf color of the hybrid between *L. xarkwrightii* 'Vesuvius' and *L. cognata* Maxim. were intermediate between the female and the male (Fig . 2.1). The maternal parent *L. xarkwrightii* 'Vesuvius' has sturdy stems, while the paternal parent *L. cognate* has fragile and sometimes distorted stems and leaves under greenhouse conditions. Six promising hybrids were selected according to their attractive ornamental phenotypes (Table 2.5 and Fig. 2.2 to 2.6).

As far as intergeneric hybridization, 36 combinations of reciprocal crossings (19 paired reciprocals) and 12 one-direction crossings between *Lychnis* and *Silene* species were attempted (Table 2.6). Among the 19 pairs of reciprocal crossings, 1 pair produced seeds in both directions (Code No. 79 & 80, the combination of *L. cognata* and *S. armeria* L.), 10 pairs produced seeds in one direction, and the others had no seed set. One out of the 12 one-direction intergeneric crossings had seed set with all three hybridizations (Code No. 157). However, no seeds germinated for any of these intergeneric combinations, so morphological traits could not be checked to ensure these seeds were from hybridizations rather than from selfing. Related information in Table 2.2 indicates that the rate of DI, DF, UI, and UR out of total seed (include DR, DI, DF, UI, and UR) is 98.63% (summary of combinations with Code No. 13, 79, 80, 110, 116, 119, 120, 128, 130, 140, 142, 157 & 173), which means there was a high possibility that the

seeds were produced from hybridization. The other part of the list in Table 2.6 is hybridization among *Silene* species. No successful crossing was observed.

In total, 86 combinations of reciprocal crossings among *Lychnis* species (43 pairs) were made (Table 2.7). Twenty six pair hybridizations had seed set in both directions (60.5%), around 80% of which differ in their successful crossing rate between the two directions. Seven pairs out of the 26, produced F_0 seeds with germination success in both directions (Code No. 1, 2; 3, 4; 60, 61; 71, 72; 75, 76; 81, 82 & 114, 115), and seeds of two pairs germinated in one direction although they had F_0 seeds in the reciprocal direction. Even in the hybrid with a similar successful crossing rate with reciprocal direction, the F_0 seeds showed morphology difference. For example, the combination *L. miqueliana* Rohrb. and *L. xhaageana* Lem. mixed hybrids (Code No. 100 & 101) had successful crossing rates of 50% and 40% for the two crossing directions, respectively (Table 2.7). The seed morphology data in Table 2.2 showed that if *L. miqueliana* was the materrnal parent, there were 27.4% of the seeds with undeveloped or had irregular shape, but if *L. miqueliana* served as the pollen donor, all seeds were undeveloped or irregular shape. Four pairs did not succeed in producing seed in either direction (9.3%) (Code No. 21, 22; 55, 56; 67, 68 & 86, 87), and the rest of the 13 pairs resulted in F_0 seeds in one direction (30.2%) (Table 2.7).

Double Flower Attempt on *L. cognata*

Lychnis species all have single flowers except the cultivar *L. flos-cuculi* 'Jenny' which has double flowers. The genus *Dianthus* L. which is within the same family with *Lychnis* has many double flower cultivars commercially. A natural mutant of *L. cognata* having more petals than five, which is typical for *Lychnis*, was used for double flower parental material by selfing or crossing with other *Lychnis* species (some crossings are mixed in the list of Tables B.1 and B.3). The progenies had either single flowers or flowers with more petals. The plants with more petal flowers seemed to be instable because they produced asymmetric flowers, but the extra petal trait

could be inherited. Seeds from these extra petal flowers were collected to facilitate further selection and breeding. Crossings between *Lychnis* and a *Dianthus* species was tried to see if hybrids could be generated between the two genera, but no seeds were produced through these crossings (not shown). Hybridations were limited, so the data were not enough to conclude if this is a feasible way to get a double flower gene in *Lychnis* from *Dianthus*.

Crossings with Mutants

Artificial mutated species *L. chalcedonica* and *L. flos-cuculi* were either crossed or selfed (Table B.2 and B.3). No promising progenies were observed.

Selections

During the hybridization process, selection among the experimental *Lychnis* population was also conducted. Three novel *Lychnis* accessions were selected. The one shown in Fig. 2.7 has not been identified taxonomically. The plant showed up in the experimental population in the second year (summer 2011) in the greenhouse. The flowers resemble *L. wilfordii* (Regel) Maxim., *L. chalcedonica*, and *L. xhaageane*. It could be a natural mutant or hybridized naturally, and is sterile. The other two were found in summer 2011, from *L. xhaageana* mixed hybrids in the second generation of selfed populations (plants obtained from selfed seeds in the list of Table B.3). One accession possessed flowers with a different color and a reflexed corolla (Fig. 2.8). Artificially selfed seeds were saved. The other *L. xhaageana* accession had two different colored flowers on one cyme (Fig. 2.9), and unfortunately proved to be sterile.

DISCUSSION

The common name of the family Caryophyllaceae is carnation family or pink family. Among the *Lychnis* species collected for this project, pink was not the prevailing color. Two species, *L. miqueliana* and *L. cognate*, are pink and have relatively big flowers, which make them outstanding in the experimental population, but they both have fragile stems and leaves when cultivated in our greenhouse .

In the selected hybrids (Table 2.4), 10 out of 17 generated from *L. cognata* were pink. Most importantly, those pink flower hybrid plants were as healthy as the other parental material. As showed in Fig. 2.1, the stem color of the hybrid between *L. xarkwrightii* 'Vesuvies' and *L. cognata* were partially inherited from 'Vesuvies' showing reddish internodes. The leaves and stems were stronger than *L. cognata*. Caryophyllaceae is an anthocyanin-containing family, so the pigment change might be because the hybrids have an intermediate amount of anthocyanin between the two parents. Anthocyanin is beneficial to living organisms due to its protection function (Hwang et al. 2011), so the hybridization may have transferred disease resistance. Also, the extra petal plants were found in *L. cognata*. Through selfing, hybridization, and selection, healthy pink flowers were obtained along with inheriting the extra petal gene(s) from *L. cognata*. The interspecific hybridizations between *L. cognata* and other *Lychnis* species were successful. As far as plant breeding, the potential good parent materials are likely the one that look undesirable, such as *L. cognata* in this project with obvious unhealthy phenotypes with fragile and distorted stems and leaves under our normal cultication conditions in greenhouses.

Lychnis miqueliana is the only species having entire petals while lacking appendages on the petals. All other traits are the same as other *Lychnis* species including five petals, fruit capsules, five stigmas, 10 anthers, connate sepals, and gynophores. *Lychnis miqueliana* did not readily produce hybrids with other species. Among 28 combinations (Table A.1) with *L. miqueliana* as

one parent, only one combination produced seeds that germinated. *Lychnis chalcedonica* is a species with a very high selfing rate, so emasculation is suggested to ensure a desirable hybridization rate. No hybrids were obtained from *L. flos-cuculi* in the hybridizations. It was observed that *L. wilfordii* maintains a very low selfing rate in the greenhouses.

This research indicates that the greenhouse environment played a big role in hybrid seed germination. We did tissue culture to successfully rescue immature hybrid seeds. Godo et al., (2009) tried interspecific crossing between autotriploid *L. senno* (2n=3x=36) with six other species (2n=24) in the genus *Lychnis*. Immature seeds were rescued and cultured in vitro, resulting in hybrids that would not have otherwise developed (Godo et al., 2009). There were many hybrid seeds in this project that did not germinate in the greenhouses that may have germinated had they been rescued and cultured in vitro. The results of this research showed that seed morphology does reflect the parental hybridization accessibility. Through seed shape data, we could speculate some seeds without germination in this project resulted from hybridization rather than selfing. So for specific hybridization combinations, including tissue culture to promote greater germination of mature or immature embryos would be desirable.

For facilitating crossing when pollen was lacking, *Lychnis* pollen was collected into a tube and stored at 5 °C. Pollens grains were good for 1 to 2 months for hybridization. Several crossings were lacking in numbers because pollen storage for long term use failed in this research. Daniel (2011) conducted pollen storage experiment for yam, and revealed that yam pollen stored at -80 °C remained viable for over 2 years. So, if equipment is available, different pollen storage method could be tried for meeting different bloom seasons.

Data in reciprocal crossings strongly indicated that cytoplasm inheritance exists in *Lychnis*, which led to different hybridization success rates, different seed germination rates, different hybrid traits, and different seed morphology for reciprocal hybridizations.

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Table 2.1 *Lychnis, Silene,* and *Dianthus* species and cultivars used in hybridization.

Category	Taxa	Variety or Cultivar
Genera	*Lychnis*	
	L. chalcedonica	
		L. chalcedonica var. *alba*
		L. chalcedonica 'White'
		L. chalcedonica 'Rauhreif'
		L. chalcedonica 'Carnea'
		L. chalcedonica 'Burning Love'
	L. cognata	
	L. miqueliana	
	L. wilfordii	
	L. flos-cuculi	
		L. flos-cuculi 'White Robin'
	L. xhaageana mixed hybrids	
		L. xhaageana 'Molten Lava'
		L. xhaageana 'Lumina Salmon'
		L. xhaageana 'Lumina Orange'
		L. xhaageana 'Lumina Brozeleaf Red'
	L. xarkwrightii	
		L.xarkwrightii 'Vesuvius'
		L. xarkwrightii 'Orange Genome'
Genera	*Silene*	
	S. armeria	
	S. pendula	
	S. plankii	
	S. acaulis ssp. *acaulescens*	
	S. delavayi	
Genera	*Dianthus*	
	Dianthus cultivars	
Mutants	*Lychnis*	
	L. chalcedonica	
	L. flos-cuculi	

Table 2.2 *Lychnis* crossings with seeds produced without seed germination.

Code No.[z]	Maternal parent	Pollen source	Total crossings	Crosses with seeds	Total seeds	**Seed morphology[y]**				
						DR	DI	DF	UI	UR
5	*L.chalcedonica*	*L. cognata*	8	4	213	0	184	0	29	0
6	*L. cognata*	*L.chalcedonica*	8	3	81	0	81	0	0	0
7	*L.chalcedonica*	*L.miqueliana*	5	3	176	13	0	0	163	0
8	*L.miqueliana*	*L.chalcedonica*	2	2	49	0	2	0	47	0
9	*L.chalcedonica*	*L. vesuvius*	11	2	38	0	0	0	38	0
10	*L. vesuvius*	*L.chalcedonica*	20	17	630	3	473	95	59	0
11	*L.chalcedonica*	*L. xhaageana* mixed hybrids	19	10	391	0	36	0	355	0
12	*L. xhaageana* mixed hybrids	*L.chalcedonica*	8	6	162	0	137	0	25	0
13	*L.chalcedonica*	*S.armeria*	6	3	37	3	0	0	17	17
15	*L.chalcedonica*	*L. xhaageana* ‘Molten Lava’	6	1	39	2	0	0	37	0
16	*L. xhaageana* ‘Molten Lava’	*L.chalcedonica*	5	4	106	0	106	0	0	0
19	*L.chalcedonica* 'Rauhreif'	*L. cognata*	5	2	31	0	0	0	31	0
23	*L.chalcedonica* 'Rauhreif'	*L. vesuvius*	6	2	15	2	0	0	13	0
24	*L. vesuvius*	*L.chalcedonica* 'Rauhreif'	6	5	200	24	116	0	60	0
26	*L. chalcedonica* 'Carnea'	*L. cognata*	7	5	180	36	144	0	0	0
28	*L.chalcedonica* 'Carnea'	*L. vesuvius*	8	4	108	27	18	0	63	0

29	*L. vesuvius*	*L.chalcedonica* 'Carnea'	7	5	123	1	120	0	2	0
31	*L. xhaageana* mixed hybrids	*L.chalcedonica* 'Carnea'	5	4	119	0	114	0	5	0
33	*L.chalcedonica* 'Burning Love'	*L. vesuvius*	7	2	50	0	0	0	50	0
39	*L.chalcedonica* 'White'	*L. cognata*	12	2	9	2	4	0	3	0
40	*L. cognata*	*L.chalcedonica* 'White'	2	1	18	0	14	0	4	0
42	*L.miqueliana*	*L.chalcedonica* 'White'	1	1	19	15	2	0	2	0
43	*L.chalcedonica* 'White'	*L. vesuvius*	11	4	69	0	0	0	69	0
44	*L. vesuvius*	*L.chalcedonica* 'White'	5	3	36	0	21	15	0	0
46	*L. wilfordii*	*L.chalcedonica* 'White'	2	2	38	6	6	0	26	0
47	*L. chalcedonica* 'White'	*L. xhaageana* mixed hybrids	8	1	24	0	0	0	24	0
48	*L. xhaageana* mixed hybrids	*L.chalcedonica* 'White'	6	3	42	3	13	0	26	0
53	*L.chalcedonica* var. *alba*	*L. cognata*	6	1	3	0	0	0	3	0
54	*L. cognata*	*L.chalcedonica* var. *alba*	2	1	9	0	6	0	3	0
57	*L.chalcedonica* var. *alba*	*L. vesuvius*	8	1	39	0	0	0	39	0
69	*L. cognata*	*L.miqueliana*	8	7	141	0	119	17	5	0
70	*L.miqueliana*	*L. cognata*	5	3	124	1	119	0	4	0
74	*L. wilfordii*	*L. cognata*	7	4	75	10	53	7	5	0
79	*L. cognata*	*S. plankii*	8	2	8	0	0	0	8	0
80	*S. plankii*	*L. cognata*	7	6	232	0	172	0	15	45
83	*L. flos-cuculi*	*L. miqueliana*	15	2	4	0	0	0	4	0

85	*L. wilfordii*	*L. flos-cuculi*	6	2	15	0	0	0	2	13
88	*L. flos-cuculi* 'White Robin'	*L. vesuvius*	6	2	6	4	0	0	2	0
89	*L. vesuvius*	*L. flos-cuculi* 'White Robin'	7	1	13	0	0	13	0	0
92	*L. xhaageana* mixed hybrids	*L. flos-cuculi* 'White Robin'	2	1	5	0	5	0	0	0
96	*L. miqueliana*	*L. vesuvius*	6	4	101	2	97	0	2	0
97	*L. vesuvius*	*L.miqueliana*	5	5	76	0	21	2	53	0
99	*L. wilfordii*	*L.miqueliana*	20	12	254	117	80	0	57	0
100	*L.miqueliana*	*L. xhaageana* mixed hybrids	8	4	96	71	19	0	6	0
101	*L. xhaageana* mixed hybrids	*L.miqueliana*	10	4	64	0	29	12	23	0
102	*L.miqueliana*	*L. xhaageana* 'Lumina Salmon'	1	0	49	0	49	0	0	0
103	*L. xhaageana* 'Lumina Salmon'	*L.miqueliana*	4	3	83	0	58	0	25	0
104	*L.miqueliana*	*L. xarkwrightii*	2	2	87	0	86	0	1	0
110	*S.plankii*	*L. miqueliana*	3	3	102	0	48	0	9	45
111	*L. vesuvius*	*L. flos-cuculi*	7	1	12	1	0	0	11	0
112	*L. vesuvius*	*L. wilfordii*	3	2	6	3	3	0	0	0
113	*L. wilfordii*	*L. vesuvius*	9	5	22	8	8	0	6	0
116	*L. vesuvius*	*S.armeria*	7	1	3	0	0	0	3	0
119	*S. plankii*	*L. vesuvius*	6	2	60	0	52	0	8	0

120	*L. vesuvius*	*S.pendula*	6	3	32	2	8	0	22	0
122	*L. wilfordii*	*L.chalcedonica*	7	1	26	0	0	0	26	0
123	*L. wilfordii*	*L.chalcedonica* var. *alba*	6	5	80	10	7	23	40	0
124	*L. wilfordii*	*L. xhaageana*	6	4	54	22	28	0	4	0
125	*L. xhaageana*	*L. wilfordii*	3	2	32	27	0	0	2	3
126	*L. wilfordii*	*L. xhaageana* mixed hybrids	8	5	43	29	3	0	10	1
128	*L. wilfordii*	*S.armeria*	11	2	2	0	0	0	2	0
130	*L. wilfordii*	*S.pendula*	8	3	31	0	0	0	27	4
135	*L. xhaageana*	*L.chalcedonica* 'Carnea'	2	2	67	0	39	0	28	0
136	*L. xhaageana*	*L.chalcedonica* 'White'	2	2	78	0	78	0	0	0
139	*L. xhaageana*	*L.miqueliana*	7	5	74	16	27	29	2	0
140	*L. xhaageana*	*S.armeria*	6	1	3	0	0	0	3	0
142	*L. xhaageana*	*S.pendula*	6	5	42	0	17	0	17	8
144	*L. xhaageana* mixed hybrids	*L.chalcedonica* 'Rauhreif'	1	1	11	0	10	0	1	0
145	*L. xhaageana* mixed hybrids	*L.chalcedonica* 'Burning Love'	1	1	7	0	5	0	2	0
151	*L. xhaageana* ‘Molten lava’	*L.chalcedonica* 'White'	2	2	89	0	87	0	2	0
152	*L. xhaageana* ‘Molten lava’	*L.chalcedonica* var. *alba*	1	1	24	1	23	0	0	0
154	*L. xhaageana* ‘Molten Lava’	*L. flos-cuculi* 'White Robin'	1	1	3	0	3	0	0	0

156	*L. miqueliana*	*L. xhaageana* ‘Molten Lava’	4	4	135	73	54	0	8	0
157	*L. xhaageana* ‘Molten Lava’	*S. pendula*	3	3	41	0	0	41	0	0
159	*L. xhaageana* 'Lumina Orange'	*L. chalcedonica* 'Carnea'	1	1	12	0	12	0	0	0
161	*L. xhaageana* 'Lumina Orange'	*L.miqueliana*	2	1	3	0	3	0	0	0
163	*L. xarkwrightii* 'Orange Genome'	*L.miqueliana*	5	2	77	1	76	0	0	0
173	*L. cognata*	*S. pendula*	9	5	74	4	0	0	70	0

[z]Coincides with that in Appendix B.1

[y]Represents seed size as developed (D), undeveloped (U), regular seed shape (R), irregular seed shape (I), and if the seeds are obviously flat (F).

Table 2.3 *Lychnis* crossings including hybrid seed germination and seed morphology.

Code No.[z]	Maternal parent	Pollen source	crossing #	# germinated	Total germination rate	Total seed	**Seed morphology[y]**				
							DR	DI	DF	UI	UR
1	*L.chalcedonica*	*L.chalcedonica* 'White'	5	5	22.0%	309	215	88	0	6	0
2	*L.chalcedonica* 'White'	*L.chalcedonica*	8	6	84.8%	361	361	0	0	0	0
3	*L.chalcedonica*	*L.chalcedonica* var. *alba*	5	4	77.8%	162	157	0	0	5	0
4	*L.chalcedonica* var. *alba*	*L.chalcedonica*	6	5	79.4%	253	253	0	0	0	0
17	*L.chalcedonica* 'Rauhreif'	*L.chalcedonica* 'Carnea'	1	1	47.0%	32	32	0	0	0	0
18	*L.chalcedonica* 'Rauhreif'	*L.chalcedonica* 'Burning Love'	4	4	38.2%	297	297	0	0	0	0
37	*L.chalcedonica* 'White'	*L.chalcedonica* 'Carnea'	6	5	82.5%	220	209	0	0	11	0
38	*L.chalcedonica* 'White'	*L.chalcedonica* 'Burning Love'	2	2	91.8%	105	105	0	0	0	0
51	*L.chalcedonica* var. *alba*	*L.chalcedonica* 'Carnea'	1	1	11.0%	37	37	0	0	0	0
52	*L.chalcedonica* var. *alba*	*L.chalcedonica* 'Burning Love'	4	1	33.3%	3	0	0	3	0	0
58	*L. vesuvius*	*L.chalcedonica* var. *alba*	8	1	0.8%	131	1	122	2	6	0
60	*L.chalcedonica* var. *alba*	*L. xhaageana* mixed hybrids	13	1	1.7%	58	2	0	0	56	0
61	*L. xhaageana* mixed hybrids	*L.chalcedonica* var. *alba*	10	1	0.9%	110	6	92	4	8	0
66	*L.chalcedonica*	*L.chalcedonica* 'Carnea'	6	5	77.4%	315	190	92	0	33	0
71	*L. cognata*	*L. vesuvius*	7	3	77.1%	109	66	0	0	43	0

72	*L. vesuvius*	*L. cognata*	12	11	70.5%	480	456	2	0	22	0
75	*L. cognata*	*L. xhaageana* mixed hybrids	10	7	65.5%	292	192	86	0	14	0
76	*L. xhaageana* mixed hybrids	*L. cognata*	12	6	82.1%	213	199	1	3	10	0
81	*L.cognata*	*L. xhaageana* 'Molten Lava'	5	4	53.3%	77	50	14	0	13	0
82	*L. xhaageana* 'Molten Lava'	*L. cognata*	4	3	87.3%	204	201	0	0	3	0
114	*L. vesuvius*	*L. xhaageana* mixed hybrids	5	2	60.6%	84	84	0	0	0	0
115	*L. xhaageana* mixed hybrids	*L. vesuvius*	8	7	68.2%	274	212	5	1	56	0
137	*L. xhaageana*	*L. cognata*	8	5	69.5%	245	224	2	0	19	0
155	*L. xhaageana* 'Molten Lava'	*L.miqueliana*	5	1	1.8%	225	3	121	21	80	0
158	*L. xhaageana* 'Molten Lava'	*L. xhaageana* mixed hybrids	1	1	92.0%	24	23	0	0	1	0
160	*L. xhaageana* 'Lumina Orange'	*L. cognata*	1	1	68.0%	25	20	0	0	5	0
167	*L.xarkwrightii* 'Vesuvius'	*L. cognata*	4	4	66.0%	176	173	0	0	3	0
168	*L.xhaageana* 'Lumina Brozeleaf Red'	*L. cognata*	1	1	64.0%	11	11	0	0	0	0

[z]Coincides with that in Table B.1.

[y]Seed size as developed (D), undeveloped (U), regular seed shape compared to parents (R), irregular seed shape (I), and if the seeds are obviously flat (F).

Table 2.4 Flower colors of selected *Lychnis* hybrids and their parents.

Code no.[z]	Maternal color	Maternal parent	Parternal color[y]	Pollen source	Hybrid color[y]
58	30B	*L.vesuvius*	white	*L.chalcedonica* var. *alba*	33B
60	white	*L.chalcedonica* var. *alba*	44B/30B[x]	*L.xhaageana* mixed hybrids	white
61	44B/30B[x]	*L.xhaageana* mixed hybrids	white	*L.chalcedonica* var. *alba*	43A
71	37A/37B	*L.cognata*	30B	*L.vesuvius*	30A; 30B; 31C
72	30B	*L.vesuvius*	37A/37B	*L.cognata*	30A; 30B; 31C; 32A; 32B; 33B; 40D; 41C
75	37A/37B	*L.cognata*	44B/30B[x]	*L.xhaageana* mixed hybrids	39A; 40B; 40D; 43A; 43B
76	44B/30B[x]	*L.xhaageana* mixed hybrids	37A/37B	*L.cognata*	32B; 33C; 39A; 40A; 43C
81	37A/37B	*L.cognata*	44B	*L.xhaageana* 'Molten Lava'	43B; 43C; 40D
82	44B	*L.xhaageana* 'Molten Lava'	37A/37B	*L.cognata*	43A
114	30B	*L.vesuvius*	44B/30B[x]	*L.xhaageana* mixed hybrids	33B; 47A
115	44B/30B[x]	*L.xhaageana* mixed hybrids	30B	*L.vesuvius*	32A; 33A; 33B; 40A
137	30B/44B[x]	*L.xhaageana*	37A/37B	*L.cognata*	30B; 32A; 33B; 40D; 41C; 43A
155	44B	*L.xhaageana* 'Molten Lava'		*L.miqueliana*	40D
158	44B	*L.xhaageana* 'Molten Lava'	44B/30B[x]	*L.xhaageana* mixed hybrids	32A; 43A
167	30B	*L.xarkwrightii* 'Vesuvius'	37A/37B	*L.cognata*	30A

[z]Coincides with Table B.1.

[y]Flower color recorded according to The Royal Horticultural Society Color Chart.

[x]Flower colors in this species are with many variations.

Table 2.5 Morphological assessments on selected *Lychnis* hybrids.

Code No.[z]	Hybridization	Corolla diameter (cm)[y]	Petal length (cm)[y]	Petal width (cm)[y]	Flower color[x]	Leaf color[x]
58	*L. vesuvius*[w]	5.80 a	2.76 a	2.14 a	33A	147A
	L.chalcedonica var. *alba*[v]	1.80 c	0.84 c	0.70 c	white	146A
	hybrids	3.66 b	1.78 b	1.82 b	33B	147B
	$LSD_{0.05}$	0.32	0.19	0.25		
60	*L. chalcedonica* var. *alba*[w]	1.80 b	0.84 b	0.70 b	white	146A
	L. xhaageana mixed hybrids[v]	4.22 a	1.96 a	1.78 a	44B	147A
	hybrids	1.28 c	0.58 c	0.48 c	white/56D	146C
	$LSD_{0.05}$	0.40	0.18	0.14		
61	*L.xhaageana* mixed hybrids[w]	4.22 a	1.96 a	1.78 a	44B	147A
	L.chalcedonica var. *alba* [v]	1.80 c	0.84 c	0.70 c	white	146A
	hybrids	3.00 b	1.58 b	1.48 b	43A	144A
	$LSD_{0.05}$	0.35	0.20	0.17		
72	*L.vesuvius*[w]	5.80 a	2.76 a	2.14 a	30B	147A
	L.cognata [v]	5.22 b	2.34 b	1.94 a	37A/37B	146A/147B
	hybrids	5.56 ab	2.58 ab	2.06 a	40D/30A/30B	137B
	$LSD_{0.05}$	0.38	0.22	NS		
81	*L.cognata*[w]	5.22 b	2.34 b	1.94 b	37A/37B	146A/147B

	L. xhaageana ‘Molten Lava’[v]	4.60 c	2.24 b	2.02 b	44B	147A/147B
	hybrids	6.00 c	2.76 a	2.74 a	43B/43C/40D	146A/147A
	$LSD_{0.05}$	0.40	0.18	0.31		
82	*L. xhaageana* ‘Molten Lava’[w]	4.60 b	2.24 b	2.02 b	44B	147A/147B
	L. cognata [v]	5.22 a	2.34 b	1.94 b	37A/37B	146A/147B
	hybrids	5.66 a	2.64 a	2.38 a	33A/33B/34A/43A/43B/43C	146A/147B
	$LSD_{0.05}$	0.58	0.27	0.34		

[z]Coincides with that in Table B.1.

[y]Note: Different letters in one column indicate means significantly different at $P \leq 0.05$, n=5.

[x]Flower and leaf color recorded according to The Royal horticultural Society Color Chart.

[w]Maternal parents.

[v]Paternal parents.

Table 2.6 Intergeneric hybridization between *Lychnis* and *Silene*, and interspecific hybridization among *Silene*.

Code No.[z]	Maternal parent	Pollen source	Crossing #	Successful crossing #	Success rate[y]
Inter-generic hybridization between *Lychnis* and *Silene* (reciprocal direction)					
13	*L. chalcedonica*	*S. armeria*	6	3	50%
14	*S. armeria*	*L. chalcedonica*	7	0	0%
35	*L. chalcedonica* 'Burning Love'	*S. armeria*	6	0	0%
36	*S. armeria*	*L. chalcedonica* 'Burning Love'	1	0	0%
62	*L. chalcedonica* var. *alba*	*S. armeria*	6	0	0%
63	*S. armeria*	*L. chalcedonica* var. *alba*	6	0	0%
64	*L. chalcedonica* var. *alba*	*S. pendula*	5	0	0%
65	*S. pendula*	*L. chalcedonica* var. *alba*	6	0	0%
77	*L. cognata*	*S. armeria*	8	0	0%
78	*S. armeria*	*L. cognata*	9	0	0%
79	*L. cognata*	*S. plankii*	8	2	25%
80	*S. plankii*	*L. cognata*	7	6	86%
105	*L. miqueliana*	*S. armeria*	2	0	0%
106	*S. armeria*	*L. miqueliana*	7	0	0%
107	*L. miqueliana*	*S. pendula*	4	0	0%
108	*S. pendula*	*L. miqueliana*	5	0	0%

109	*L. miqueliana*	*S. plankii*	5	0	0%
110	*S. plankii*	*L. miqueliana*	3	3	100%
116	*L. vesuvius*	*S. armeria*	7	1	14%
117	*S. armeria*	*L. vesuvius*	11	0	0%
118	*L. vesuvius*	*S. plankii*	5	0	0%
119	*S. plankii*	*L. vesuvius*	6	2	33%
120	*L. vesuvius*	*S. pendula*	6	3	50%
121	*S. pendula*	*L. vesuvius*	6	0	0%
128	*L. wilfordii*	*S. armeria*	11	2	18%
129	*S. armeria*	*L. wilfordii*	1	0	0%
130	*L. wilfordii*	*S. pendula*	8	3	38%
131	*S.pendula*	*L. wilfordii*	5	0	0%
132	*L. wilfordii*	*S. plankii*	5	0	0%
133	*S. plankii*	*L. wilfordii*	5	0	0%
140	*L. xhaageana*	*S. armeria*	6	1	17%
141	*S. armeria*	*L. xhaageana*	8	0	0%
142	*L. xhaageana*	*S. pendula*	6	5	83%
143	*S. pendula*	*L. xhaageana*	5	0	0%
149	*L. xhaageana* mixed hybrids	*S. armeria*	14	0	0%

150	*S. armeria*	*L. xhaageana* mixed hybrids	19	0	0%
172	*S. pendula*	*L. cognata*	3	0	0%
173	*L. cognata*	*S. pendula*	9	5	56%
Inter-generic hybridization between *Lychnis* and *Silene* (one direction)					
49	*L. chalcedonica* 'White'	*S. armeria*	6	0	0%
50	*L. chalcedonica* 'White'	*S. pendula*	4	0	0%
94	*L. flos-cuculi* 'White Robin'	*S. plankii*	5	0	0%
146	*L. xhaageana* mixed hybrids	*S. plankii*	1	0	0%
147	*L. xhaageana* mixed hybrids	*S. pendula*	5	0	0%
148	*L. xhaageana* mixed hybrids	*S. acaulis* ssp. *acaulescens*	2	0	0%
157	*L. xhaageana* 'Molten Lava'	*S. pendula*	3	3	100%
166	*L. xarkwrightii* 'Orange Genome'	*S. armeria*	3	0	0%
169	*S. armeria*	*L. chalcedonica* 'Carnea'	6	0	0%
170	*S. armeria*	*L. flos-cuculi*	2	0	0%
171	*S. pendula*	*L. chalcedonica*	5	0	0%
177	*S. delavayi*	*L. xarkwrightii*	1	0	0%
Hybridization among *Silene*					
174	*S. pendula*	*S. armeria*	5	0	0%
175	*S. pendula*	*S. plankii*	3	0	0%

176	*S. plankii*	*S. armeria*	1	0	0%

[z]Coincides with that in Table B.1. Two consecutive numbers in the same colume alternating with another two in adjacent column indicate reciprocal hybridizations.

[y]No seeds has germinated for any combination with success rate within this table.

Table 2.7 Hybridizations among *Lychnis* species with reciprocal crossings.

Code no.[z]	Maternal parent	Pollen source	Crossing #	# of Successfull crosses	Success rate	Seed germination
1	*L.chalcedonica*	*L.chalcedonica* 'White'	5	5	100%	Y
2	*L.chalcedonica* 'White'	*L.chalcedonica*	8	6	75%	Y
3	*L.chalcedonica*	*L.chalcedonica* var. *alba*	5	4	80%	Y
4	*L.chalcedonica* var. *alba*	*L.chalcedonica*	6	5	83%	Y
5	*L.chalcedonica*	*L. cognata*	8	4	50%	N
6	*L. cognata*	*L.chalcedonica*	8	3	38%	N
7	*L.chalcedonica*	*L.miqueliana*	5	3	60%	N
8	*L.miqueliana*	*L.chalcedonica*	2	2	100%	N
9	*L.chalcedonica*	*L. vesuvius*	11	2	18%	N
10	*L. vesuvius*	*L.chalcedonica*	20	17	85%	N
11	*L.chalcedonica*	*L. xhaageana* mixed hybrids	19	10	53%	N
12	*L. xhaageana* mixed hybrids	*L.chalcedonica*	8	6	75%	N
15	*L.chalcedonica*	*L. xhaageana* ‘Molten Lava’	6	1	17%	N
16	*L. xhaageana* ‘Molten Lava’	*L.chalcedonica*	5	4	80%	N
19	*L.chalcedonica* 'Rauhreif'	*L. cognata*	5	2	40%	N
20	*L. cognata*	*L.chalcedonica* 'Rauhreif'	1	0	0%	-

21		*L.chalcedonica* 'Rauhreif'	*L.miqueliana*	1	0	0%	-
22		*L.miqueliana*	*L.chalcedonica* 'Rauhreif'	5	0	0%	-
	23	*L.chalcedonica* 'Rauhreif'	*L. vesuvius*	6	2	33%	N
	24	*L. vesuvius*	*L.chalcedonica* 'Rauhreif'	6	5	83%	N
26		*L.chalcedonica* 'Carnea'	*L. cognata*	7	5	71%	N
27		*L. cognata*	*L.chalcedonica* 'Carnea'	3	0	0%	-
	28	*L.chalcedonica* 'Carnea'	*L. vesuvius*	8	4	50%	N
	29	*L. vesuvius*	*L.chalcedonica* 'Carnea'	7	5	71%	N
30		*L.chalcedonica* 'Carnea'	*L. xhaageana* mixed hybrids	3	0	0%	-
31		*L. xhaageana* mixed hybrids	*L.chalcedonica* 'Carnea'	5	4	80%	N
	33	*L.chalcedonica* 'Burning Love'	*L. vesuvius*	7	2	29%	N
	34	*L. vesuvius*	*L.chalcedonica* 'Burning Love'	1	0	0%	-
39		*L.chalcedonica* 'White'	*L. cognata*	12	2	17%	N
40		*L. cognata*	*L.chalcedonica* 'White'	2	1	50%	N
	41	*L.chalcedonica* 'White'	*L.miqueliana*	6	0	0%	-
	42	*L.miqueliana*	*L.chalcedonica* 'White'	1	1	100%	N
43		*L.chalcedonica* 'White'	*L. vesuvius*	11	4	36%	N
44		*L. vesuvius*	*L.chalcedonica* 'White'	5	3	60%	N
	45	*L.chalcedonica* 'White'	*L. wilfordii*	3	0	0%	-

	46	*L. wilfordii*	*L.chalcedonica* 'White'	2	2	100%	N
47		*L.chalcedonica* 'White'	*L. xhaageana* mixed hybrids	8	1	13%	N
48		*L. xhaageana* mixed hybrids	*L.chalcedonica* 'White'	6	3	50%	N
	53	*L.chalcedonica* var. *alba*	*L. cognata*	6	1	17%	N
	54	*L. cognata*	*L.chalcedonica* var. *alba*	2	1	50%	N
55		*L.chalcedonica* var. *alba*	*L.miqueliana*	18	0	0%	-
56		*L.miqueliana*	*L.chalcedonica* var. *alba*	3	0	0%	-
	57	*L.chalcedonica* var. *alba*	*L. vesuvius*	8	1	13%	N
	58	*L. vesuvius*	*L.chalcedonica* var. *alba*	8	6	75%	Y
60		*L.chalcedonica* var. *alba*	*L. xhaageana* mixed hybrids	13	2	15%	Y
61		*L. xhaageana* mixed hybrids	*L.chalcedonica* var. *alba*	10	8	80%	Y
	67	*L. cognata*	*L.flos-cuculi*	7	0	0%	-
	68	*L.flos-cuculi*	*L. cognata*	6	0	0%	-
69		*L. cognata*	*L.miqueliana*	8	7	88%	N
70		*L.miqueliana*	*L. cognata*	5	3	60%	N
	71	*L. cognata*	*L. vesuvius*	7	4	57%	Y
	72	*L. vesuvius*	*L. cognata*	12	11	92%	Y
73		*L. cognata*	*L. wilfordii*	5	0	0%	-
74		*L. wilfordii*	*L. cognata*	7	4	57%	N

	75	*L. cognata*	*L. xhaageana* mixed hybrids	10	8	80%	Y
	76	*L. xhaageana* mixed hybrids	*L. cognata*	12	6	50%	Y
81		*L. cognata*	*L. xhaageana* ‘Molten Lava’	5	5	100%	Y
82		*L. xhaageana* ‘Molten Lava’	*L. cognata*	4	4	100%	Y
	84	*L.flos-cuculi*	*L. wilfordii*	7	0	0%	-
	85	*L. wilfordii*	*L.flos-cuculi*	6	2	33%	N
86		*L.flos-cuculi*	*L. xhaageana* mixed hybrids	2	0	0%	-
87		*L. xhaageana mixed hybrids*	*L.flos-cuculi*	3	0	0%	-
	88	*L.flos-cuculi* 'White Robin'	*L. vesuvius*	6	2	33%	N
	89	*L. vesuvius*	*L.flos-cuculi* 'White Robin'	7	1	14%	N
91		*L.flos-cuculi* 'White Robin'	*L. xhaageana* mixed hybrids	7	0	0%	-
92		*L. xhaageana* mixed hybrids	*L.flos-cuculi* 'White Robin'	2	1	50%	N
	96	*L.miqueliana*	*L. vesuvius*	6	4	67%	N
	97	*L. vesuvius*	*L.miqueliana*	5	5	100%	N
98		*L.miqueliana*	*L. wilfordii*	6	0	0%	-
99		*L. wilfordii*	*L.miqueliana*	20	12	60%	N
	100	*L.miqueliana*	*L. xhaageana* mixed hybrids	8	4	50%	N
	101	*L. xhaageana* mixed hybrids	*L.miqueliana*	10	4	40%	N
102		*L.miqueliana*	*L. xhaageana* 'Lumina Salmon'	1	0	0%	N

103		*L. xhaageana* 'Lumina Salmon'	*L.miqueliana*	4	3	75%	N
	112	*L. vesuvius*	*L. wilfordii*	3	2	67%	N
	113	*L. wilfordii*	*L. vesuvius*	9	5	56%	N
114		*L. vesuvius*	*L. xhaageana* mixed hybrids	5	2	40%	Y
115		*L. xhaageana* mixed hybrids	*L. vesuvius*	8	8	100%	Y
	124	*L. wilfordii*	*L. xhaageana*	6	4	67%	N
	125	*L. xhaageana*	*L. wilfordii*	3	2	67%	N
126		*L. wilfordii*	*L. xhaageana* mixed hybrids	8	5	63%	N
127		*L. xhaageana* mixed hybrids	*L. wilfordii*	4	0	0%	-
	155	*L. xhaageana* 'Molten Lava'	*L.miqueliana*	5	5	100%	Y
	156	*L.miqueliana*	*L. xhaageana* 'Molten Lava'	4	4	100%	N
163		*L. xarkwrightii* 'Orange Genome'	*L.miqueliana*	5	2	40%	N
164		*L.miqueliana*	*L. xarkwrightii* 'Orange Genome'	1	0	0%	-

[z]Coincides with that in Table B.1.

[z]Two consecutive numbers in the same colume alternating with another two in adjacent column indicate reciprocal hybridizations.

Fig. 2.1 Hybrid (middle) between *L. xarkwrightii* 'Vesuvius' (left, female) and *L. cognata* (right, male). Hybrid has intermediate flower color, stem color, and leaf health (Code No. 167 as indicated in Table 2.2 and 2.3).

Fig. 2.2 *Lychnis vesuvius* (left), *L. chalcedonica* var. *alba* (right) and hybrid in the middle (code No. 58).

Fig. 2.3 *Lychnis chalcedonica* var. *alba* (left), *L. xhaageana* mixed hybrids (right), and hybrid in the middle (Code No. 60).

Fig. 2.5 *Lychnis vesuvius* (left), *L. cognata* (right) and hybrid in the middle (Code No. 72)

Fig. 2.4 *Lychnis xhaageana* mixed hybrids (left), *L.chalcedonica* var. *alba* (right), and hybrid in the middle (Code No. 61)

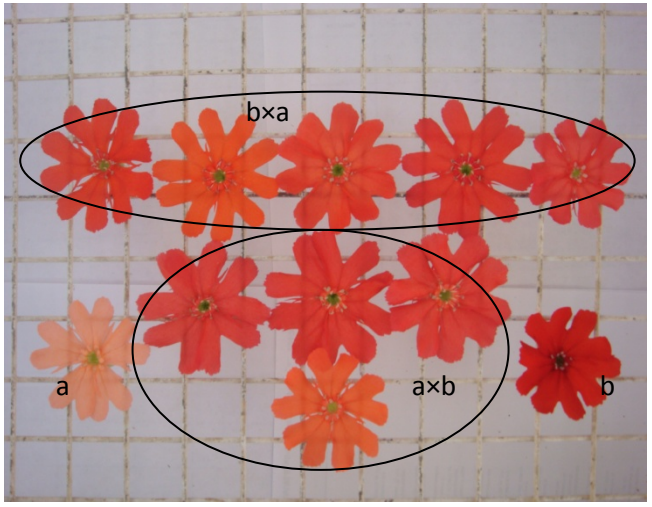

Fig. 2.6 *Lychnis cognata* (a), *L. xhaageana* 'Molten Lava' (b), and hybrids (a×b and b×a) (Code No. 81 and 82)

Fig. 2.7 A natural mutant or naturally hybridized flower (sterile).

(a) From left to right, *L. wilfordii*, the selection (species unidentified), *L. xhaageana*, and *L. chalcedonica*.

(b) The selection (species unidentified)

Fig. 2.8 Reflexed corolla (a) individual flower (b) inflorescences (*L. xhaageana* mixed hybrids)

Fig. 2.9 Different color flowers on the same inflorescence (*L. xhaageana* mixed hybrids; sterile)

CHAPTER III

GENETIC MANIPULATION

INTRODUCTION

The ornamental value of the genus *Lychnis L.* was recognized commercially by horticulturalists, yet has not been well exploited. Induced mutagenesis, which has a high mutation frequency, has been used to create new cultivars in plant breeding. Induced mutagenesis has its profound meaning not only in producing desired mutants for new cultivars, but also potentially providing specific mutants for genetics study. The effects of different mutagens are also an important subject for related fields, since different mutagens have different effects on DNAs or RNA due to their specific mutative mechanism (Snustad and Simmons, 2006).

Ethyl methansulfanate (EMS), a common mutagen, is known for effective alkylation of guanine bases on DNA, which results in A-T base pair substitution for G-C pair during the next round of duplication causing point mutations. Caffeine causes chromosome aberration and sister chromatid exchange in mammalian cell (Bittueva et al., 2007). Its mechanism on plants is not yet so clear, and no plant cultivar has been reported through caffeine mutation. Caffeine was selected as a mutagen to test along with EMS in this experiment due to its non-toxic nature to humans, and because of its mutation effects on animals.

This research was designed both for mutation breeding and to evaluate the effects of different mutagens on *Lychnis* phenotypic traits.

LITERATURE REVIEW

The purpose of inducing mutants in ornamentals is to get variegation in the leaves, develop new flower colors, and alter plant growth habit. Artificial mutations could be induced by physical radiation, chemical mutagens, as well as somaclonal mutations in tissue culture. Ethyl Methanesulfonate (EMS) is an effective and efficient chemical mutagen that acts by alkylating DNA and is possibly a carcinogenic chemical (Aaron and Lee, 1978). Ethyl methanesulfonate has been used in medical research on animals for a long time. Sega (1974) firstly connected EMS with sex-linked DNA through studying repair of germ cell DNA after treatment with EMS on male mice. A relationship was determined between the dosage of EMS and recessive lethals induced in sperm cells of *Drosophila melanogaster* (Aaron and Lee, 1978). Ethyl methanesulfonate has been used on a number of different plant species. For example, Alcantara et al., (1996) produced several novel foliage mutants of *Capsicum annuum* L. using EMS, and Predieri (2001) released an apple (*Malus domestica* Borkh.) cultivar named 'Belrene' with the specific trait of fruit earliness in 1970 through EMS mutation breeding.

No research on *Lychnis* mutation work has been reported. Our preliminary trial showed that some species of *Lychnis* are EMS sensitive. Two mutant results were observed. One is chlorophyll chimeras on leaves. Other crops where chlorophyll mutants have been reported with the use of EMS include peas, carrots, soybeans, lentils, radishes, and barley (Miller et al. 1984; Harten, 1998). The other visible effect of EMS on *Lychnis* is weaker pollen and stronger stigmas than found in non-mutated flowers.

Caffeine as a mutagen on animal cells has also been studied (Kuhlmann et al., 1968; Kramata et al., 2005). It can result in chromosome aberration and sister chromatid exchange in mammalian cell cultures in vitro (Bittueva et al., 2007). Caffeine as a pre- or post- treatment along with other mutagens was studied in plant mutations (Swietlin et al., 1973; Zhu et al., 1995). Chen et al.

(2000) analyzed two types of somatic meiosis-like reduction in *Vicia faba* L. induced at a high rate by treating germinated seeds in caffeine solutions. Caffeine should be tested as an efficient chemical mutagen as it is friendlier to the environment and humans than EMS.

Conditions such as mutagen concentration, treatment time, and temperature are all important factors that affect success of mutation breeding. A tendency at present, which is thought to be a more desirable mutation protocol, is to treat with lower mutagen concentrations or lower doses to get a survival rate of 70-80%, than in the past which corresponded to a survival rate of about 50% (Harten, 1998).

Mutation breeding is also a systematic scheme. The purpose of mutation breeding is to directly create cultivars or create new germplasm for further breeding. It is clear that only a few mutations have a chance to survive, as a mutant trait may have a deletious effect on plant survival. In fact, many favorable mutations induced somewhere in a plant may be lost due to improper screening (Vainstein, 2002), while other mutations may not surface until the progeny of the mutated plant (M2 plants) are grown out (Harten, 1998).

MATERIALS AND METHODS

Three replications of 100 seeds of *Lychnis coronaria* (L.) Desr., *L. xhaageana* Lem. 'Molten Lava', *L. chalcedonica* L., and *L. flos-cuculi* L. were placed separately in coffee filters (Brew Rite®). Seeds obtained from various sources (Appendix A, Table A.4) were then soaked in different chemical mutagens for 24 hours at 15 °C in a growth chamber (Nor-Lake, Hudson, WI).

Chemical mutagens included ethyl methanesulfanate (EMS) (Acros Organics, New Jersey) and 200 mg tablets of Jet-Alert caffeine (Bell Pharmaceuticals, Minneapolis, MN). Mutagen treatments included 0.6% EMS (v/v), 10% caffeine (w/v), 20% caffeine (w/v), 0.6% EMS plus 10% caffeine, 0.6% EMS plus 20% caffeine, and a control using only deionized water. Deionized water was used as the solvent for all mutagens.

After soaking in the mutagen solutions, seeds were rinsed three times for 2 minutes under tap water then planted in 20 cm pots (Itml, Middlefield, OH). The planting media was Metro-Mix 702 (Sun Gro Horticulture, Vancouver, Canada). All treatments and replications for each species were completely randomized in the Oklahoma State University Horticultural Research Greenhouses, Stillwater**.** Media was watered as needed. This experiment was conducted from March to August, 2011. The temperatures in greenhouses were set at 21 °C daytime and 18 °C during night, and actual temperatures varied with weather conditions.

Seed vigor, seedling surviving, number of mutants, as well as M1 morphologic variation were recorded for evaluating mutagens and their influence on *Lychnis* M1 seedlings. Seedlings were counted daily after germination until the germination rates of most treatments were stable for three consecutive days. Seed vigor was determined by mean germination time (MGT) (Matthews et al., 2011).

$$MGT=\sum (f\cdot x) \ / \sum x$$

x, the newly germinated seeds on the f^{th} day; f, the number of days since the planting day; $\sum x$, total number of germinated seeds. Seedling heights were measured 70 days after planting. Natural heights (canopy height) were measured for *L. coronaria* and *L. flos-cuculi*, and straight heights (stem length) for *L. xhaageana* 'Molten Lava' and *L. chalcedonica*. Leaf chlorophyll content was measured using a SPAD-502 chlorophyll meter (Spectrum Technologies, Plainfield, Illinois).

Flowering days are the days from planting date (Mar. 11^{th}) to flowering date. The mutation rate was calculated based on the last day of the germination number (rate) recorded for the seedling surviving dynamics. Mutants for each replication of every treatment were counted after all other quantity data were measured. Seedling numbers for each treatment and replication were counted 10 days apart, beginning with the date that germination rates were stable for 3 consecutive days to

trace seedling surviving dynamics. Data were analyzed by SAS/STAT® software using ANOVA procedure. All percentage data were transformed by arcsinn $\sqrt{\chi}$ before analysis.

RESULTS

L.coronaria

Ethyl methanesulfanate and EMS + 20 % caffeine dwarfed plant height of *L. coronaria* significantly at $P \leq 0.05$ level compared to the control, whereas 10% caffeine, 20% caffeine, and EMS + 10% caffeine did not show significant effects on plant height for this species (Table 3.1). All treatments did not change seed vigor (mean germination time) and leaf chlorophyll content of *L. coronaria*. Flower time difference was not recorded, since this species does not flower without vernalization under greenhouse conditions. Seeding survival rates of *L. coronaria* had no difference during the observation period (Fig. 3.1 (a)). The species *L. coronaria* had no mutants observed until the experiment ended (Table 3.5), whereas mutants of this species were observed in a former mutagenesis treatment with the same mutagen rate for a longer time (Fig. 3.1).

L. xhaageana 'Molten Lava'

Results showed that caffeine induced mutants in *L. xhaageana* 'Molten Lava'. Significant differences were found ($P \leq 0.05$) within the caffeine treatments or EMS plus caffeine treatments for mean germination time, plant height, and flower date (Table 3.2). All treatments except EMS treatment delayed mean germination time for *L. xhaageana* 'Molten Lava', which means seed vigor was lowered during the germination process. For plant height, all treatments except 10% caffeine were significant. All EMS plus caffeine treatments, both high and low rate caffeine, delayed the flowering date and extended the vegetative growth period significantly for *L. xhaageana* 'Molten Lava'. No chlorophyll difference was detected. Only the EMS plus 20% caffeine treatment for *L. xhaageana* 'Molten Lava' had a statistically significant mutant rate. The EMS, EMS plus 10% caffeine, and EMS plus 20% caffeine treatments dramatically lowerd

seeding survival rate of *L. xhaageana* 'Molten Lava' one month later compared to the other three treatments after the germination rate was stable for at least three consective days (Fig. 3.1). The EMS plus 20% caffeine treatment further decreased seedling survival rate on May 22, 2011 (Fig. 3.1 (b)). Fig. 3.3 shows the dwarfed plants with smaller flowers. *Lychnis xhaageana* 'Molten Lava' is rich with pigment in the calyxes, while the mutants in Fig. 3.3 are lacking reddish color in calyxes. As for plant pigments, the other mutant found in this species showed blended spots on the red petals (not shown). Plus, all the mutants had abnormal stamens.

L. chalcedonica

Lychnis chalcedonica is EMS sensitive. Every treatment with EMS incurred differences in comparison to mean germination time, plant height, chlorophyll content, and flowering data, yet caffeine only treatments had no significant effects (Table 3.3). The same was also observed for mutation rate (Table 3.5). The EMS, EMS plus 10% caffeine, and EMS plus 20% caffeine treatments had longer effect on seeding survival rate of *L. chalcedonica*. One month after recording began (seedling survival rate started from germination rate stable for consecutive 3 days), these treatments obviously killed more seedling than other treatments that contained no EMS (Fig. 3.1 (c)), and the longer the time lasted, the stronger the effects were observed. Several exotic mutants were obtained through mutagenesis for *L. chalcedonica*, which included leaf shape and color variation (Fig. 3.4-3.7). Stamen abnormality due to mutagenesis was also found (Fig. 3.4) as the case reported for *L. xhaageana* 'Molten Lava'.

L. flos-cuculi

For *L. flos-cuculi*, all treatments with EMS had effects on plant height and leaf chlorophyll content making it an EMS sensitive species. However, 10% caffeine treatments decreased mean germination time, which means caffeine caused stronger seed vigor than other treatments including control (Table 3.4). EMS plus 20% caffeine effectively affected mutation rate of *L.*

flos-cuculi (Table 3.5). The flowering dates of species *L. flos-cuculi* were not analylized since the heat during summer inhibited flowering, making the data inaccurate to reflect the effects of mutation. All treatments, except 10% caffeine, had no effect on *L. flos-cuculi*'s seedling survival rates. The 10% caffeine treatment surprisingly had a significant increase in seedling survival rate a month after germination rate stable for consecutive 3 days (Fig. 3.1 (d)). Mutagenesis caused leaf texture, shape, and color variation in *L. flow-cuculi* (Fig. 3.8).

DISCUSSION

Mean germination time is an index showing how fast the seed lot germinates, and reflects seed vigor. The longer the mean germination time lasts, the lower the seed vigor is observed. Using this index, each species had a different reaction with the mutagen treatments. *Lychnis coronaria* had no difference on any treatment; *L. xhaageana* 'Molten Lava' had decreased seed vigor by both the caffeine only and the EMS plus caffeine treatments; *L. chalcedonica* incurred lower seed vigor only with treatments with EMS; whereas in the caffeine only treatments, *L. flos-cuculi* had higher seed vigor than the control and any other treatments that were with EMS. In fact, any mutagens that contained EMS did not markedly change the mean germination time of *L. flos-cuculi*.

Mutagenisis causes DNA or nucleotide changes, so perhaps it incurs more DNA repair or other mechanism that prolong the DNA replication process, hence generally increasing mean germination time as shown in species *L. xhaageana* 'Molten Lava' and *L. chalcedonica*. Zhu et al., (1995) reported that caffeine as a post treatment agent lowered the mutation frequency of EMS treatment on soybean, so as for the increased seed vigor in *L. flos-cuculi*, caffeine may have facilitated the DNA repair in the seeds, since the germination rate of this species had decreased notably since a preliminary experiment was conducted one year prior this research. The effect

and efficiency of certain kinds of mutagen might relate to both a difference in species seed morphology and seeds deteriation conditions.

Mean germination time (MGT) and seedling survival rate reflect seed quality in different aspects, and one can not mask the others characteristic. Lower rate of caffeine not only shortened the mean germination time (MGT) of *L. flos-cuculi*, but also increased the seedling survival rate of this species. The two indexes showed that low rate caffeine substantially improved *L. flos-cuculi*'s seed quality, in essence reversing the natural deterioration. The results theoretically coinside with Zhu et al. (1995) report about caffeine's post-treatment function for EMS mutagensis .

The phenotypical mutants in this research were obtained more from the EMS plus caffeine treatments than the EMS only treatments. Caffeine mutated seedlings, with half a leaf becoming notable lighter green than the other half, were observed when *L. flos-cuculi* plants grew to the 4 leaf stage, than this trait disappeared. There were no mature mutated plants produced by caffeine only treatments in this research, so mutagenesis might require higher caffeine concentration or longer treatment duration than which was set in this experiment. The rate of 20% caffeine is the maximum value which could be made using this kind of caffeine tablets, which is a much higher amount of caffeine (20 mM which is around 3.88 g/L) than was used as a post treatment to reserve the effects of EMS on soybean (Zhu et al., 1995). The use of caffeine as a mutagen should be tested further.

There were several mutants with half leaf color change as mentioned above for *L. flos-cuculi* and *L. chalcedonica* seedlings (Fig. 3.6 (b)). Other leaf color change involved border color variation, such in *L. coronaria*, *L. chalcedonica*, and *L. flos-cuculi (*Fig. 3.2, 3.6a, and 3.8b*)*. A mutant *L. flos-cuculi* plant putatively enhanced its drought tolerance through leaf structure change together with leaf color change which is shown in Fig. 3.8 (b).

Ornamental traits are traditionally transmitted through crossing, yet there has been successful trait transfer through molecular breeding in certain ornamental cultivars (Casanova et al., 2005). Wadl et al., (2010) found that two genes epistastically regulate dogwood leaf colors. With the development of ornamental genetic engineers, more visual traits related to genes will be discovered, which would in turn direct ornamental breeding for target characteristics. Further confirmation will be required in long term for the putative theory that different genes mediate different parts of colors on *Lychnis* leaves, which could potentially provide theoretical basis for ornamental molecular breeding.

Delayed flower time was recorded for both *L. chalcedonica* and *L. xhaageana* 'Molten Lava'. Muthusamy et al. (2011) reported that a lower mutagenic treatment rate induced early flowering in cotton (*Gossypium hirsutum* L.); however, a higher rate resulted in delayed flowering. An early fruiting apple (*Malus domestica* Borkh.) cultivar was also induced by EMS (Predieri, 2001), which is supported by the enhancement of agronomical traits promoted by low rate mutagenesis on cotton (Muthusamy et al., 2011). Based on our experiment, *Lychnis flos-cuculi* is a species that can flower the first year under greenhouse conditions. However, it was observed that over 20 plants among *L. flos-cuculi* mutants had not flowered in the greenhouses for two years (observation on mutants obtained in preliminary trial conducted 2010). Büttner et al. (2010) revealed EMS mutated an unknown loci located on chromosome Ⅳin sugar beet (*Beta vulgaris* L.) beside the bolting gene *B* located on chromosome II, and the two genes both have an effect on flower time. Hohmann et al. (2005) has reported an efficient EMS protocol for getting non-bolting mutated sugar beets from an early bolting sugar beet which was without vernilization requirement, and obtained some mutant lines that required vernalization for flowering. The non-flowering *L. flos-cuculi* mutants are being tested to see if vernalization could induce flowering. The research and exertion of the flower time change function could benefit the plant production

for the purpose of extending vegetative growth, or shorten the breeding circulation by early maturity effects.

Bigger flowers were observed on *L. chalcedonica* mutants (Fig. 3.4), and smaller flowers were seen along with stunted plants in mutant *L. xhaageana* plants (Fig. 3.5). There were no flower color change or variegation on these selected species expect there were some bleached spots on *L.xhaageana* 'Molten Lava' petals which did not appear to be a good trait.

The obvious changes on flowers was that mutagenesis caused stronger pistils and weaker stamens in *L. chalcedonica* and *L. xhaageana* 'Molten Lava', which has also been reported to cause mutations in the reproduction system in animals (Sega, 1974; Aaron and Lee, 1978). Taking advantage of *L. chalcedonica* mutants which have less pollen or no pollen as females in breeding might facilitate hybridization since based on our observation, this species have a high self pollination rate.

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Table 3.1 Mean germination time, plant height, and leaf chlorophyll content in response to different chemical mutagen treatments for *Lychnis coronaria.*

Mutagen	Mean germination time (days)[z]	Plant height (cm)[zy]	Chlorophyll[zy]
Control	15.94 ± 2.04 a	7.69 ± 0.55 a	33.19 ± 1.60 a
0.6% EMS	19.53 ± 2.41 a	4.10 ± 0.41 b	31.94 ± 3.11 a
10% Caffeine	16.63 ± 1.00 a	7.36 ± 0.80 a	34.76 ± 2.54 a
20% Caffeine	17.70 ± 0.54 a	7.54 ± 0.50 a	30.37 ± 2.53 a
0.6 % EMS+ 10% Caffeine	20.04 ± 2.43 a	6.01 ± 0.87 a	31.96 ± 1.77 a
0.6% EMS+ 20% Caffeine	17.52 ± 0.45 a	4.10 ± 1.92 b	28.33 ± 2.32 a
LSD 0.05	NS	1.75	NS

[z]Different letters in one column indicate means significantly different at $P \leq 0.05$; n=3.

[y]Readings taken from a SPAD chlorophyll meter, unitless.

Table 3.2 Mean germination time, plant height, and leaf chlorophyll content in response to different chemical mutagen treatments for *Lychnis xhaageana.*

Mutagen	Mean Germination Time (days)[z]	Plant Height (cm)[z]	Chlorophyll[zy]	Flowering date (days)[z]
Control	11.29 ± 0.92c	12.23 ± 1.28a	27.34 ± 3.12a	74.67 ± 1.53b
0.6 % EMS	12.31 ± 0.48bc	2.19 ± 0.49c	23.79 ± 2.52a	106.00 ± 3.00ab
10% Caffeine	13.48 ± 0.93ab	10.57 ± 1.25ab	26.64 ± 4.02a	77.67 ± 3.21b
20% Caffeine	14.29 ± 2.10a	9.86 ± 1.23b	28.46 ± 2.68a	77.67 ± 2.08b
0.6 % EMS+ 10% Caffeine	13.61 ± 0.29ab	2.03 ± 1.63c	27.98 ± 1.63a	116.33 ± 11.59a
0.6% EMS+ 20% Caffeine	14.24 ± 0.64a	1.39 ± 0.52c	26.15 ± 2.15a	136.00 ± 41.39a
LSD 0.05	1.90	2.04	NS	31.43

[z]Different letters in one column indicate means significantly different at $P \leq 0.05$; n=3.

[y]Readings taken from a SPAD chlorophyll meter, unitless.

Table 3.3 Mean germination time, plant height, and leaf chlorophyll content in response to different chemical mutagen treatments for *Lychnis chalcedonica.*

Mutagen	Mean germination Time (days)[z]	Plant height (cm)[z]	Chlorophyll[zy]	Flowering date (days)[z]
Control	9.54 ± 0.37b	26.08 ± 1.45a	27.53 ± 0.19a	71.33 ± 2.08c
0.6 % EMS	11.09 ± 0.46a	9.59 ± 2.26b	19.41 ± 1.45b	90.33 ± 1.53a
10% Caffeine	9.92 ± 0.39b	24.87 ± 2.90a	26.60 ± 1.78a	70.33 ± 2.52c
20% Caffeine	9.95 ± 0.73b	26.05 ± 7.16a	27.70 ± 2.11a	68.67 ± 1.53c
0.6 % EMS+ 10% Caffeine	11.01 ± 0.16a	11.06 ± 1.92b	19.85 ± 0.75b	83.00 ± 6.24b
0.6% EMS+ 20% Caffeine	11.46 ± 0.82a	8.54 ± 2.46b	20.12 ± 1.79b	89.00 ± 1.73a
LSD 0.05	0.96	6.36	2.67	5.50

[z]Different letters in one column indicate means significantly different at $P \leq 0.05$; n=3.

[y]Readings taken from a SPAD chlorophyll meter, unitless.

Table 3.4 Mean germination time, plant height, and leaf chlorophyll content in response to different chemical mutagen treatments for *Lychnis flos-cuculi.*

Mutagen	Mean germination time (days)[z]	Plant height (cm)[z]	Chlorophyll[zy]
Control	14.56 ± 0.69ab	10.21 ± 0.34a	36.88 ± 2.25a
0.6 % EMS	15.83 ± 0.53a	6.65 ± 0.04b	32.37 ± 2.86b
10% Caffeine	12.47 ± 0.75c	9.27 ± 0.85a	36.66 ± 2.09a
20% Caffeine	13.59 ± 0.86bc	9.90 ± 0.78a	37.77 ± 1.05a
0.6 % EMS+ 10% Caffeine	15.85 ± 1.18a	6.70 ± 0.83b	32.01 ± 1.10b
0.6% EMS+ 20% Caffeine	14.88 ± 1.13ab	6.78 ± 1.09b	34.94 ± 2.70ab
LSD 0.05	1.58	1.32	3.79

[z]Different letters in one column indicate means significantly different at $P \leq 0.05$; n=3.

[y]Reading taken from a SPAD chlorophyll meter, unitless.

Table 3.5 Mutation rate (%) of four tested *Lychnis* species.

Mutagen	*L. coronaria*[z]	*L. xhaageana*[z]	*L. chalcedonica*[z]	*L. flos-cuculi*[z]
Control	0.00	0.00 d	0.00 b	0.00 b
0.6 % EMS	0.00	26.67 bc	9.92 a	12.42 b
10% Caffeine	0.00	0.00 d	0.00 b	0.00 b
20% Caffeine	0.00	1.71 cd	0.00 b	4.32 b
0.6 % EMS+ 10% Caffeine	0.00	42.22 b	6.10 a	7.04 b
0.6% EMS+ 20% Caffeine	0.00	88.89 a	11.46 a	34.17 a

[z]In columns, data followed by lower case letters indicate significant difference at $P \leq 0.05$; n=3.

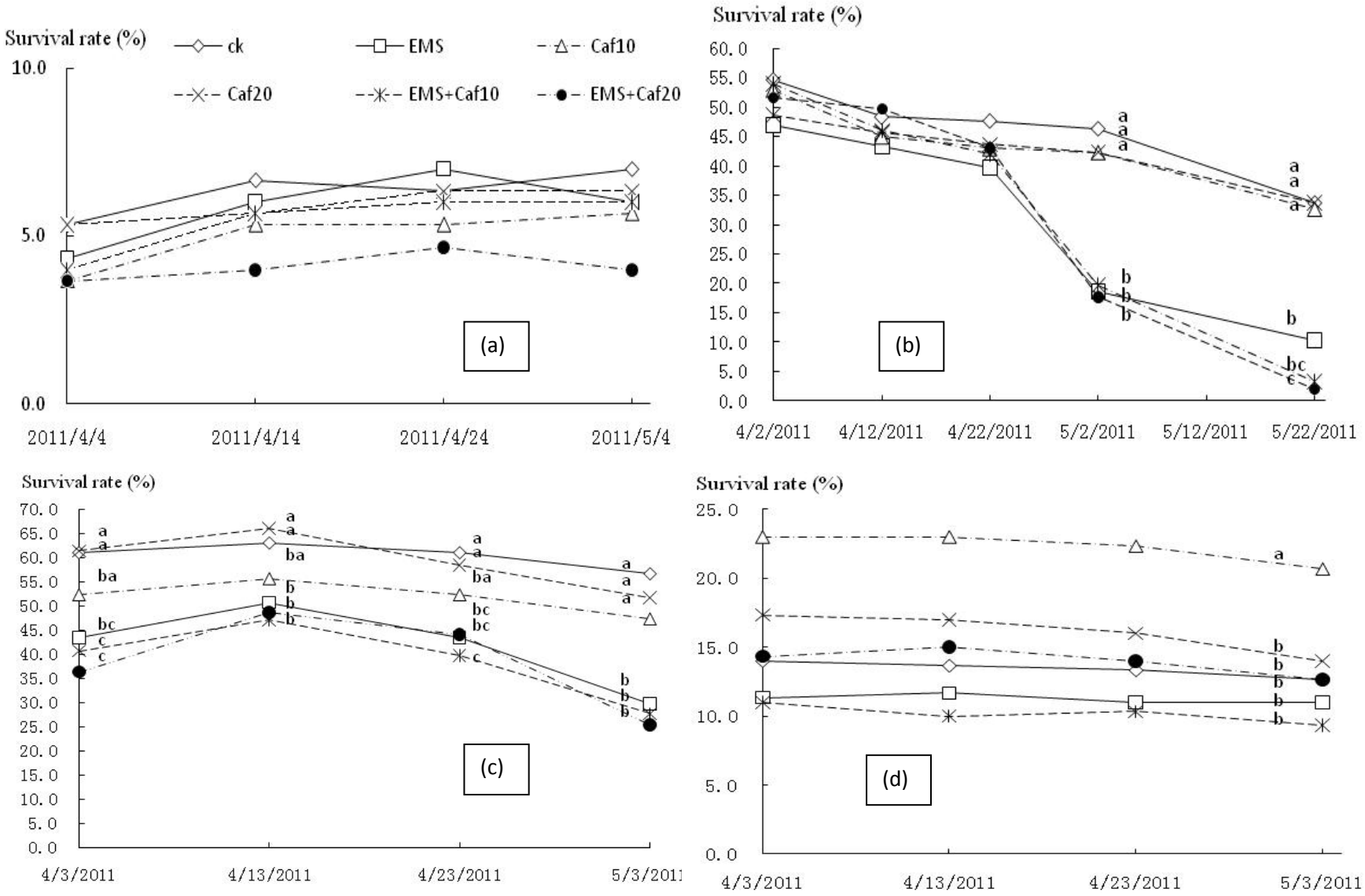

Fig. 3.1 Seedling survival dynamics after germination rate being stable for three consecutive days. Different letters on the same date denote seedling survival rate different at $P \leq 0.05$. (a) *L. coronaria*, (b) *L. xhaageana*, (c) *L. chalcedonica*, and (d) *L. flos-cuculi*.

Fig. 3.2 A *L. coronaria* mutant (left) and a normal *L. coronaria* (right).

Fig. 3.3 Mutated *L. xhaageana* ‘Molten Lava’ (front, dwarfed statures and smaller flowers) and normal *L. xhaageana* ‘Molten Lava’ (back, higher plants).

Fig. 3.4 A mutant *L. chalcedonica* flower (right) compared with a normal flower (left). The mutant has more lobes on the petal edges, broader petals, a thicker pistil, and no stamens.

Fig. 3.5 A selected mutant of *L. chalcedonica* with more flowers blooming simultaneously and earlier (left) than the normal *L. chalcedonica* (right). Most of those flowers were either selfed of crossed, but failed to set seed.

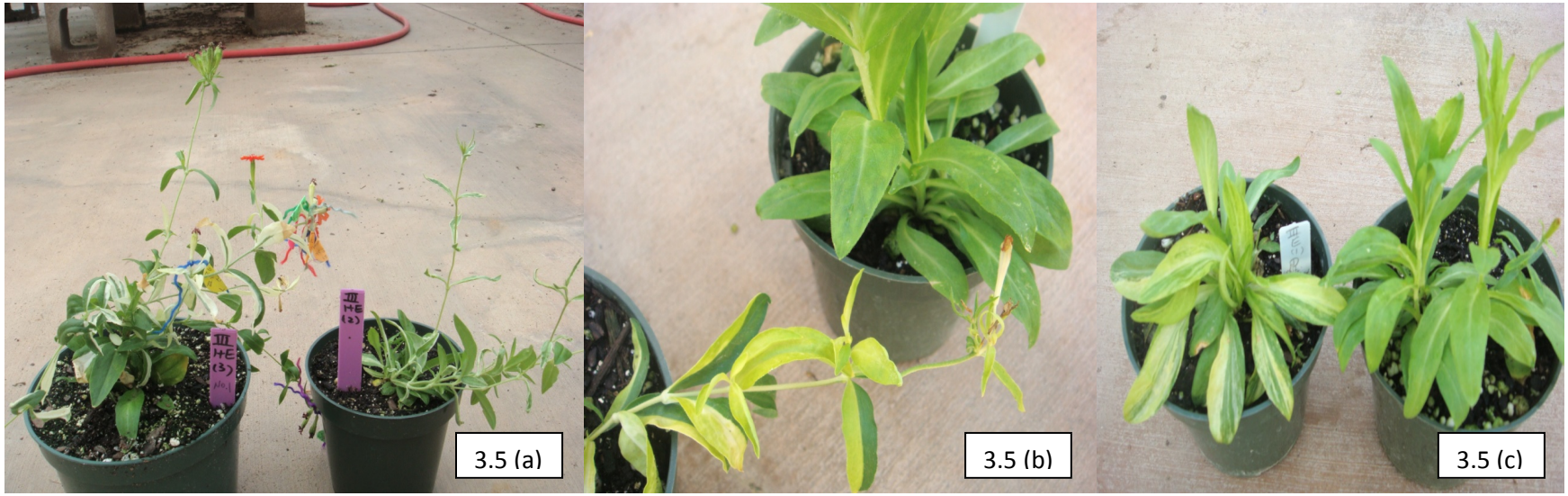

Fig. 3.6 Leaf variegation of *L. chalcedonica* (a) Leaves with white variegation (left) and leaves with white borders (right). (b) Leaves with light green variegation (left) compared with normal leaves (right). (c) Leaves with stripes of green and light green colors (left) and normal leaves (right).

Fig. 3.7 Mutated leaves of *L. chalcedonica* with folded leaves.

Fig. 3.8 Mutations of *L. flos-cuculi* compared with normal plants (circled).

CHAPTER IV

ASEXUAL PROPAGATION

INTRODUCTION

Asexual propagation is an important technique in the horticulture industry for mass production of ornamentals. Many ornamentals are commercially propagated by cuttings, which is an easy and cost effective propagation method. There are many factors that affect rooting, such as accurate control of moisture, temperature, light, hormone concentration, media, and stock plant quality. 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.

Asexual propagation is commonly used in the nursery industry (Nair and Zhang, 2010), and can produce genetically identical plants (Danehloueipour et al., 2006). *Lychnis* L. is a promising genus for extensive use as a landscape ornamental due to its drought tolerance, profuse flowering, and perennial characteristic. As species are favored by consumers, a production method will need to be established to quickly produce plants to keep up with consumer demands. Moreover, according to our observation, *Lychnis* is readily cross pollinated, so to ensure genetic identity for released cultivars, taking asexual cuttings will have to be established.

Two different cultivars were selected for the experimental materials. *Lychnis chalcedonica* L. is a well know representative in the genus *Lychnis*. *Lychnis coronaria* (L.) Desr. is morphologically and eco-physiological quite different from *L. chalcedonica*. Asexual propagation of *Lychnis* has not been reported in the literature. The purpose of this experiment is to establish a protocol for vegetatively propagating of *Lychnis*.

LITERATURE REVIEW

Vegetative propagation is an essential technique and widely used for producing stock plants. No asexual propagation methods have been developed (leaf or stem cutting) for *Lychnis* or its related genus *Silene* L. However, Chen et al. (2006) established tissue culture protocols for *L. senno* Siebold & Zucc. Developing a protocol for stem or leaf cuttings would allow for a rapid and easier way to propagate mutants or superior hybrids compared to tissue culture, especially when those plants of interest are with poor seed set and seed germination rate or need a long time to establish from seeds.

Cold storage is a regular commercial procedure on stem cuttings of *Dianthus caryophyllus* L., which is also in the Caryophyllaceaee family, to produce roots (Holley and Baker, 1990). Three cultivars were studied on the influence of cold storage and hormone treatment on rooting success. The results showed different carnation cultivars respond differently to treatments, and Garrido et al., (1996) indicated that this might be because different cultivars have different endogenous auxin levels. Research on cold storage and fresh cuttings plus exogenous auxin application were carried out on another two carnation cultivars, and results support the previous conclusions mentioned above (Garrido et al., 1998). Garrido et al. (2002) revealed that mature leaves attached on *D. caryophyllus* cuttings are essential to rooting since IAA goes from mature leaves to stems to promote root development, while cuttings with only immature leaves need 24 more days for roots initiation to allow the juvenile leaves to mature.

Effect of exogenous auxin on cuttings from other plant stocks, especially for reluctant rooting species, should be stronger than *D. caryophyllus* as that species develops roots easily. Beside hormone, other factors, namely humidity, temperature of the media, and stem age are also important for onset of roots on cuttings. Peat-perlite at a volume ratio 70:30 and mist irrigation

keeping ambient relative humidity around 85-75% worked well for carnation cuttings (Garrido et al., 1996; 1998; 2002).

MATERIALS AND METHODS

Seeds of *L. chalcedonica* and *L. coronaria* bought from J. L. Hudson, Seedsman (La Honda, California) were planted in March 2011 and May 2010 respectively, in the Oklahoma State University Horticultural Department Research Greenhouses, Stillwater. For each species, three kinds of hormones and two kinds of media were evaluated plus a control without any hormones. Hormone treatments included five second dip applications of 1,000 ppm hormone. Hormones used included HORMEX Rooting Powder (Brooker chemical, Chatsworth, CA) No. 1, DIP-N GROW liquid rooting cencertrate (DIP'N GROW, Inc. Clackamas, Oregon), and HORTUS IBA Water Soluble Salts™ (HORTUS USA CORP. New York, NY). Cuttings were stuck in perlite and vermiculite, respectively.

Each treatment had six replications with 15 cuttings per replication for *L. chalcedonica*, and four replications with 15 cuttings per replication for *L. coronaria*. All treatments were randomized under automatic mist irrigation. The irrigation models for *L. chalcedonica* were 8 seconds per minute, and 15 seconds every 32 minutes for *L. coronaria*. Asexual propagation of *L. chalcedonica* was conducted June 28th, 2011, and data collected 28 days later. *L. coronaria* was propagated on Aug. 9th, 2011, and data collected 44 days later on Sep. 22nd. The temperatures in greenhouses were set at 21 °F during daytime and 18 °F at night, and actual temperatures and light varied with weather conditions.

For *L. chalcedonica*, stem cuttings had 4 pairs of leaves, and were cut below the top two pairs of leaves from the meristem. For *L. coronaria*, cuttings were a single leaf with a small lengthwise part of stem tissue to ensure the stems included a potential axillary bud for better vegetative growth after rooting.

Data collected on *L. chalcedonica* were root number per cutting, root length, and rooting percentage per replication. For root numbers, the median number of the estimated range was recorded. If root number was estimated among the range 1-5, then it was recorded as 3; if the range was estimated among 6-10, then it was 8; if the range was estimated among 11-15, then it was recorded as 13. Data collected on *L. coronaria* include root length, rooting percentage, and root dry weight. Roots were harvested after length and percentage data were collected. Individual plant roots were put in coin envelopes then put into a Precision Scientific Oven (Jouan Inc., Winchester, VA) at 70 °C for 48 hours then weighed (Somasegaran et al., 1983). Data analyzed by SAS/STAT® software using generalized linear mixed models methods with GLIMMIX procedure.

RESULTS

L. chalcedonica

The estimated root number (Table 4.1) and rooting percentage (Table 4.4) of *L. chalcedonica* was clearly promoted by hormones, yet were not significantly changed by media factors and the interaction of media and hormones. For root number and rooting percentage, all hormone treatments were significantly better than the control, and DIP-N GROW showed the best results. The interaction of hormones and media worked effectively on root length (Table 4.2) and total root amount, which was obtained by multiplying estimated root number and root length (Table 4.3). The treatments that were notably better than the control for both root length and total root amount were vermiculite and DIP-N GROW, vermiculite and HORMEX, and perlite and HORMEX. In summary, DIP-N GROW with vermiculite, and HORMEX with both kinds of media worked well for *L. chalcedonica*.

L. coronaria

Root length, root weight, and root percentage of *L. coronaria* were all notably correlated with the interaction of media and hormones (Table 4.5, 4.6, and 4.7). Treatments of vermiculite without hormone (control), perlite with either HORTUS or DIP-N GROW significantly enhanced root length than other treatments (Table 4.5) and had the highest rooting percentage (Table 4.7). Perlite with DIP-N GROW and vermiculite with HORTUS had better root dry weight than other treatments, whereas, the root dry weight was very small in this case, and could account for the overall rooting performance. Overall, vermiculite without hormone (control) and perlite with either HORTUS or DIP-N GROW could be considered the best treatments *L. coronaria* rooting.

DISCUSSION

This experiment focused on the effect of different soil mediums and hormone products. The active ingredient for the three commercial rooting hormones are all indole-3-butyric acid (IBA), which is an effective auxin for regenerating roots for plant cuttings and plant tissue culture (Baig et al., 2011; Laubscher et al., 2008; Sharma et al., 2006). Mist irrigation was adjusted differently for the two species according to their natural physiological water needs.

A preliminary trial showed the two species were slow to initiate roots on cuttings without any hormone. The hormone treatments decreased rooting time compared with preliminary trial results and increased rooting percentage on *L. chalcedonica* compared with the control in this experiment, but the highest rooting percentage only reached 54.93% with DIP-N GROW. Besides rooting percentage, the number of roots of *L. chalcedonica* also responded positively to hormone addition. Rooting percentage is an important index in exploring the procedure for a species. Percentage of *L. coronaria* was much higher than *L. chalcedonica* and determined by the interaction of media and hormone yet reaching the maximum value 88.56% with vermiculite without hormone. The media treatments did not show an independent effect on either of the two species.

The two species clearly responded differently to the combinations of media and hormones. *Lychnis chalcedonica* favored vermiculite with DIP-N GROW, and HORMEX with both kinds of media; while the hormone HORMEX was not effective with *L. coronaria,* but this species did respond positively to perlite with DIP-N GROW or simply vermiculite without any hormone. So cutting performance was highly species related, and the interaction of media and hormone did exhibit effects on rooting of both species. The results coincided with Laubscher and Ndakidemi (2008), who found that hormone and media had an interaction effect on root induction of *Leucadendron laxum* I.Williams cuttings.

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. *Lychnis coronaria* is an obligate vernalization species for reproductive growth. It was thought that establishing an asexual propagation method for *L. coronaria* might also be an effective way to transfer vernalization or substituting vernalization requirement through manipulating environmental factors of the cuttings.

Some technical issues arose during our research and are worth noting. In a preliminary trial, *Lychnis coronaria* was able to initiate roots on leaves even without hormone as the cuttings had adequate time and appropriate environmental factors, but required further investigation on how to best develop shoots on the leaf cuttings. Hence, we developed the cuttings with less than a half longitude stem portion to ensure there was an allixary bud potentially existing between a leaf and the stem part, and by doing so, we can produce more cuttings on one plants as the plants of *L. coronaria* are tufted and without obvious arial stems in vegetative growth stage. Thus, the rooting process for *L. coronaria* was more about leaf cuttings than stem cuttings. *Lychnis coronaria* initiated roots on leaves in our preliminary trial without hormones, and the hormone DIP-N GROW also promoted roots developing on leaf blades during this experiment.

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Table 4.1 Root number of *L. chalcedonica* responding to different hormones products at 1,000 ppm.

Hormones	Mean root number [z]
DIP-N GROW	4.044 a
HORMEX	3.272 a b
HORTUS	2.611 b
CONTROL	0.883 c

[z]Different letters in the column indicate differences are significant at $P \leq 0.05$, n=90.

Table 4.2 Root length (mm) of *L. chalcedonica* due to the interaction of media and hormones at 1,000 ppm.

Media	Hormones	Mean root length (mm)[z]
Vermiculite	DIP-N GROW	32.44 a
Vermiculite	HORMEX	32.26 a
Perlite	HORMEX	19.86 b
Perlite	HORTUS	19.42 b c
Vermiculite	HORTUS	19.34 b c
Vermiculite	CONTROL	19.15 b c
Perlite	DIP-N GROW	17.44 b c
Perlite	CONTROL	7.60 c

[z]Different letters in the column indicate differences are significant at $P \leq 0.05$, n=90.

Table 4.3 Total root mass (root number × root length) of *L. chalcedonica* due to the interaction of media and hormones at 1,000 ppm.

Media	Hormones	Total root amount[z]	
Vermiculite	DIP-N GROW	352.64	a
Vermiculite	HORMEX	287.59	a b
Perlite	HORMEX	190.52	b c
Perlite	DIP-N GROW	148.14	b c d
Vermiculite	HORTUS	125.00	c d
Perlite	HORTUS	118.34	c d
Vermiculite	CONTROL	114.12	c d
Perlite	CONTROL	31.55	d

[z]Different letters in the column indicate differences are significant at $P \leq 0.05$, n=90.

Table 4.4 Rooting percentage (%) of *L. chalcedonica* due to different hormones at 1,000 ppm.

Hormones	Rooting percentage (%)[z]	
DIP-N GROW	54.93	a
HORTUS	52.81	a
HORMEX	46.04	a
CONTROL	19.40	b

[z]Different letters in the column indicate differences are significant at $P \leq 0.05$, n=6.

Table 4.5 Root length (mm) of *L. coronaria* due to the interaction of media and hormones at 1,000 ppm.

Media	Hormones	Root length (mm)[z]	
Vermiculite	CONTROL	31.67	a
Perlite	HORTUS	16.75	b
Perlite	DIP-N GROW	12.08	b c
Vermiculite	HORMEX	9.32	b c d
Vermiculite	DIP-N GROW	7.40	b c d
Vermiculite	HORTUS	1.25	c d
Perlite	HORMEX	0.13	d
Perlite	CONTROL	0.12	d

[z]Different letters in the column indicate differences are significant at $P \leq 0.05$, n=60.

Table 4.6 Root weight (g) of *L. coronaria* due to the interaction of media and hormones at 1,000 ppm.

Media	Hormones	Root weight (g)[z]	
Perlite	DIP-N GROW	0.0667	a
Vermiculite	HORTUS	0.0620	a
Vermiculite	CONTROL	0.0405	a b
Vermiculite	HORMEX	0.0387	a b
Perlite	HORTUS	0.0296	a b
Perlite	CONTROL	0.0260	a b
Vermiculite	DIP-N GROW	0.0254	a b
Perlite	HORMEX	0.0122	b

[z]Different letters in the column indicate differences are significant at $P \leq 0.05$, n=4.

Table 4.7 Rooting percentage (%) of *L. coronaria* due to the interaction of media and hormones at 1,000 ppm.

Media	Hormones	Rooting percentage (%)[z]	
Vermiculite	CONTROL	88.56	a
Perlite	HORTUS	80.87	a b
Perlite	DIP-N GROW	75.05	a b
Vermiculite	HORMEX	56.90	b
Vermiculite	DIP-N GROW	48.32	bc
Vermiculite	HORTUS	18.03	c d
Perlite	CONTROL	1.69	d
Perlite	HORMEX	0.43	d

[z]Different letters in the column indicate differences are significant at $P \leq 0.05$, n=4.

CHAPTER V

SUMMARY

Lychnis L. is a genus native to the temperate areas in the Northern Hemisphere, and has great potential for extensive use as a bedding plant in landscapes due to its attractive flowers, good drought tolerance, and winter hardness. Some species have several released cultivars, yet this genus is not broadly used in landscapes or as potted plants. Further domestication efforts are evidently required for meeting commercial needs. There has been limited interest in genetic manipulation within *Lychnis* germplasm, making the genus promising for ornamental breeding. The purpose of this research was to create new hybrids or obtain exotic materials through hybridization and artificial mutation, and explore asexual cutting protocols for selected species.

Over one thousand pollinations were made artificially in the greenhouse with hybridization aimed at intergeneric, interspecific, crossing with mutants, as well as selfed selections. Cytological inheritance of seed set rate, seed morphology, and flower color were observed. Seventeen hybrids were recorded, among which six representative hybrids were drastically different from their parental plants. The hybrids were either interspecific or intraspecific, whereas no intergeneric hybrids were generated. Some hybrid and good sterile selections were found.

As for gene manipulation, the chemical mutagens ethyl methansulfante (EMS) and caffeine were used to test the efficiency and effectiveness of the chemical mutagens on *Lychnis*. Different species responded differently to the mutagens. Caffeine showed some effects in this experiment, but needs further work to test its optimum effeciency. In summary, EMS worked well for *Lychnis*, and many mutants were selected for leaf distortion, variegation, flower size and habit

change, as well as potential drought tolerance improvement. Mutagenesis affected the reproductive organ development as some mutants were sterile.

Propagation of stem and leaf cuttings were conducted to establish asexual propagation procedures for *Lychnis*, which would be important to propagate any sterile hybrids or cultivars. Rooting abilities of the two species responded differently to the interaction of media and hormones. The best treatments for *L. chalcedonica* were vermiculite with DIP-N GROW, and HORMEX with both kinds of media. The best treatments for *L. coronaria* were perlite with DIP-N GROW or HORTUS, and simply vermiculite without any hormone.

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APPENDICES

APPENDIX A

Background tables for this research program include *Lychis* variety list, imformative data from literatures, and seed sources for this research.

Table A. 1 Examples of some common *Lychnis* cultivars.

Lychnis species	cultivars
*L.chalcedonic*a	‘Rauhreif'
	‘Carnea'
	‘Bruning Love'
	‘White'
L. xhaageana	‘Molten Lava'
	‘Lumian Salmon'
	‘Lumian Mix'
	‘Lumian Orange'
	‘Lumian Bronzeleaf Red'
L. flos- cuculi	‘White Robin'
	‘Jenny'
L. flos-jovis	‘Horts Variety'
	‘Peggy'
L. coronaria	‘Angel's Blush'
	‘Cerise'
L.viscaria	‘SnowBird’
	‘ViscaFire’
L. x arkwrightii	‘Orange Gnome’
	‘Vesuvius’
L. xwalkeri	‘Abbotswood Rose’

Table A.2 Seed set in intergeneric hybrids (Kruckeberg, 1962)

Hybrid	Crossing numbers	Seeds set in F_1	Viable F2 seeds
Lychnis drummondii × *Silene douglasii*	2	31	yes
L. drummondii × *S. grayi*	1	6	no
L. drummondii × *S. grayi*	1	2	no
L. drummondii × *S. parryi*	5	27	yes
S. parryi × *L. drummondi*	3	7	yes
L. drummondii × *S. sargentii*	1	7	no
L. drummondii × *S. scaposa*	1	6	no
S. scaposa × *L. drummondii*	1	2	no
L. drummondii × *S. scouleri*	1	18	no
S. scouleri × *L. drummondii*	1	1	no

Note: Hybrids and data selected from original table according parental genera *Lychnis* and *Silene*.

Table A.3 Chromosome number of *Lychnis* and *Silene*

Taxon	Chromosome number	References
L. miqueliana	2*n*=24	Godo et al., 2009
L. chalcedonica	2*n*=24	Godo et al., 2009
L. wilfordii	2*n*=24	Godo et al., 2009
L. sieboldii	2*n*=24	Godo et al., 2009
L. coronata	2*n*=24	Godo et al., 2009
L. viscaria	2n=24	Böcher,1977
L. alpina	2n=24	Böcher,1977
L. flos-cuculi	2n=24	Blackburn, 1924
L. flos-jovis	2n=24	Blackburn, 1924
S. pendula	2n=24	Blackburn, 1924

Table A.4 Seed sources for presented research.

Category	Taxa	Variety or Cultivar	Seed cources
Genera	*Lychnis*		
	L.chalcedonica		J. L. Hudson, Seedsman
		L. chalcedonica var. *alba*	Chiltern Seeds
		L. chalcedonica 'White'	Hardyplants.com
		L. chalcedonica 'Rauhreif'	Hardyplants.com
		L. chalcedonica 'Carnea'	Hardyplants.com
		L. chalcedonica 'Burning Love'	Hardyplants.com
	L. cognata		B and T World Seeds
	L. coronaria		J. L. Hudson, Seedsman
	L. miqueliana		Alplains
	L. wilfordii		Alplains
	L. flos-cuculi		J. L. Hudson, Seedsman
		L. flos-cuculi 'White Robin'	Hardyplants.com
	L. xhaageana	mixed hybrids	Chilternseeds
		L. xhaageana ‘Molten Lava’	Dave’s Garden
		L. xhaageana 'Lumina Salmon'	Hardyplants.com
		L. xhaageana 'Lumina Orange'	Hardyplants.com
		L. xhaageana 'Lumina Brozeleaf Red'	Hardyplants.com
	L.xarkwrightii		B and T World Seeds
		L.xarkwrightii 'Vesuvius'	Hardyplants.com
		L. xarkwrightii 'Orange Genome'	Hardyplants.com
Genera	*Silene*		
	S. armeria		Eden brothers®
	S. pendula		Eden brothers®
	S. plankii		Alplains
	S. acaulis ssp.	*acaulescens*	Alplains
	S. delavayi		Alplains
Genera	*Dianthus*		Alplains
		Dianthus cultivars	Wal-mart market

APPENDIX B

Overall hybridizations list in Appendix B.

Note: the code number in table B.1 is an index in ‘Chapter II Hybridization’ for tables and results description.

Table B.1 Intraspecific and interspecific hybridizations among *Lychnis* or *Silene*, and intergeneric hybridization between *Lychnis* and *Silene*.

Code no.	*Maternal parent*	*Pollen source*	Crossing #	Successful crossing #	Success rate	Hybrid seed germination
1	*L.chalcedonica*	*L.chalcedonica* 'White'	5	5	100%	Y
2	*L.chalcedonica* 'White'	*L.chalcedonica*	8	6	75%	Y
3	*L.chalcedonica*	*L.chalcedonica* var. *alba*	5	4	80%	Y
4	*L.chalcedonica* var. *alba*	*L.chalcedonica*	6	5	83%	Y
5	*L.chalcedonica*	*L. cognata*	8	4	50%	N
6	*L. cognata*	*L. chalcedonica*	8	3	38%	N
7	*L. chalcedonica*	*L. miqueliana*	5	3	60%	N
8	*L. miqueliana*	*L. chalcedonica*	2	2	100%	N
9	*L. chalcedonica*	*L. vesuvius*	11	2	18%	N
10	*L. vesuvius*	*L. chalcedonica*	20	17	85%	N
11	*L. chalcedonica*	*L. xhaageana* mixed hybrids	19	10	53%	N
12	*L. xhaageana* mixed hybrids	*L. chalcedonica*	8	6	75%	N
13	*L. chalcedonica*	*S. armeria*	6	3	50%	N
14	*S. armeria*	*L. chalcedonica*	7	0	0%	-
15	*L. chalcedonica*	*L. xhaageana* 'Molten Lava'	6	1	17%	N
16	*L. xhaageana* 'Molten Lava'	*L. chalcedonica*	5	4	80%	N

17	*L. chalcedonica* 'Rauhreif'	*L. chalcedonica* 'Carnea'	1	1	100%	Y
18	*L. chalcedonica* 'Rauhreif'	*L. chalcedonica* 'Burning Love'	4	4	100%	Y
19	*L. chalcedonica* 'Rauhreif'	*L. cognata*	5	2	40%	N
20	*L. cognata*	*L. chalcedonica* 'Rauhreif'	1	0	0%	-
21	*L. chalcedonica* 'Rauhreif'	*L. miqueliana*	1	0	0%	-
22	*L. miqueliana*	*L. chalcedonica* 'Rauhreif'	5	0	0%	-
23	*L. chalcedonica* 'Rauhreif'	*L. vesuvius*	6	2	33%	N
24	*L. vesuvius*	*L. chalcedonica* 'Rauhreif'	6	5	83%	N
25	*L. chalcedonica* 'Rauhreif'	*L. xhaageana*	2	0	0%	-
26	*L. chalcedonica* 'Carnea'	*L. cognata*	7	5	71%	N
27	*L. cognata*	*L. chalcedonica* 'Carnea'	3	0	0%	-
28	*L. chalcedonica* 'Carnea'	*L. vesuvius*	8	4	50%	N
29	*L. vesuvius*	*L. chalcedonica* 'Carnea'	7	5	71%	N
30	*L. chalcedonica* 'Carnea'	*L. xhaageana* mixed hybrids	3	0	0%	-
31	*L. xhaageana* mixed hybrids	*L. chalcedonica* 'Carnea'	5	4	80%	N
32	*L. chalcedonica* 'Burning Love'	*L. cognata*	3	0	0%	
33	*L. chalcedonica* 'Burning Love'	*L. vesuvius*	7	2	29%	N
34	*L. vesuvius*	*L. chalcedonica* 'Burning Love'	1	0	0%	-
35	*L. chalcedonica* 'Burning Love'	*S. armeria*	6	0	0%	-

36	*S. armeria*	*L. chalcedonica* 'Burning Love'	1	0	0%	-
37	*L. chalcedonica* 'White'	*L. chalcedonica* 'Carnea'	6	5	83%	Y
38	*L. chalcedonica* 'White'	*L. chalcedonica* 'Burning Love'	2	2	100%	Y
39	*L. chalcedonica* 'White'	*L. cognata*	12	2	17%	N
40	*L. cognata*	*L. chalcedonica* 'White'	2	1	50%	N
41	*L. chalcedonica* 'White'	*L. miqueliana*	6	0	0%	-
42	*L. miqueliana*	*L. chalcedonica* 'White'	1	1	100%	N
43	*L. chalcedonica* 'White'	*L. vesuvius*	11	4	36%	N
44	*L. vesuvius*	*L. chalcedonica* 'White'	5	3	60%	N
45	*L. chalcedonica* 'White'	*L. wilfordii*	3	0	0%	-
46	*L. wilfordii*	*L. chalcedonica* 'White'	2	2	100%	N
47	*L. chalcedonica* 'White'	*L. xhaageana* mixed hybrids	8	1	13%	N
48	*L. xhaageana* mixed hybrids	*L. chalcedonica* 'White'	6	3	50%	N
49	*L. chalcedonica* 'White'	*S. armeria*	6	0	0%	-
50	*L. chalcedonica* 'White'	*S. pendula*	4	0	0%	-
51	*L. chalcedonica* var. *alba*	*L. chalcedonica* 'Carnea'	1	1	100%	Y
52	*L. chalcedonica* var. *alba*	*L. chalcedonica* 'Burning love'	4	1	25%	Y
53	*L. chalcedonica* var. *alba*	*L. cognata*	6	1	17%	N
54	*L. cognata*	*L. chalcedonica* var. *alba*	2	1	50%	N

55	*L. chalcedonica* var. *alba*	*L. miqueliana*	18	0	0%	-
56	*L. miqueliana*	*L. chalcedonica* var. *alba*	3	0	0%	-
57	*L. chalcedonica* var. *alba*	*L. vesuvius*	8	1	13%	N
58	*L. vesuvius*	*L. chalcedonica* var. *alba*	8	6	75%	Y
59	*L. chalcedonica* var. *alba*	*L. xhaageana*	2	0	0%	-
60	*L. chalcedonica* var. *alba*	*L. xhaageana* mixed hybrids	13	2	15%	Y
61	*L. xhaageana* mixed hybrids	*L. chalcedonica* var. *alba*	10	8	80%	Y
62	*L. chalcedonica* var. *alba*	*S. armeria*	6	0	0%	-
63	*S. armeria*	*L. chalcedonica* var. *alba*	6	0	0%	-
64	*L. chalcedonica* var. *alba*	*S. pendula*	5	0	0%	-
65	*S. pendula*	*L. chalcedonica* var. *alba*	6	0	0%	-
66	*L. chalcedonica*	*L. chalcedonica* 'Carnea'	6	6	100%	Y
67	*L. cognata*	*L. flos-cuculi*	7	0	0%	-
68	*L. flos-cuculi*	*L. cognata*	6	0	0%	-
69	*L. cognata*	*L. miqueliana*	8	7	88%	N
70	*L. miqueliana*	*L. cognata*	5	3	60%	N
71	*L. cognata*	*L. vesuvius*	7	4	57%	Y
72	*L. vesuvius*	*L. cognata*	12	11	92%	Y
73	*L. cognata*	*L. wilfordii*	5	0	0%	-

74	*L. wilfordii*	*L. cognata*	7	4	57%	N
75	*L. cognata*	*L. xhaageana* mixed hybrids	10	8	80%	Y
76	*L. xhaageana* mixed hybrids	*L. cognata*	12	6	50%	Y
77	*L. cognata*	*S. armeria*	8	0	0%	-
78	*S. armeria*	*L. cognata*	9	0	0%	-
79	*L. cognata*	*S. plankii*	8	2	25%	N
80	*S. plankii*	*L. cognata*	7	6	86%	N
81	*L. cognata*	*L. xhaageana* ‘Molten Lava’	5	5	100%	Y
82	*L. xhaageana* ‘Molten Lava’	*L. cognata*	4	4	100%	Y
83	*L. flos-cuculi*	*L. miqueliana*	15	2	13%	N
84	*L. flos-cuculi*	*L. wilfordii*	7	0	0%	-
85	*L. wilfordii*	*L. flos-cuculi*	6	2	33%	N
86	*L. flos-cuculi*	*L. xhaageana* mixed hybrids	2	0	0%	-
87	*L. xhaageana* mixed hybrids	*L. flos-cuculi*	3	0	0%	-
88	*L. flos-cuculi* 'White Robin'	*L. vesuvius*	6	2	33%	N
89	*L. vesuvius*	*L. flos-cuculi* 'White Robin'	7	1	14%	N
90	*L. flos-cuculi* 'White Robin'	*L. xhaageana*	1	0	0%	-
91	*L. flos-cuculi* 'White Robin'	*L. xhaageana* mixed hybrids	7	0	0%	-
92	*L. xhaageana* mixed hybrids	*L. flos-cuculi* 'White Robin'	2	1	50%	N

93	*L. flos-cuculi* 'White Robin'	*L. miqueliana*	7	0	0%	-
94	*L. flos-cuculi* 'White Robin'	*S. plankii*	5	0	0%	-
95	*L. miqueliana*	*L. chalcedonica* 'Carnea'	1	0	0%	-
96	*L. miqueliana*	*L. vesuvius*	6	4	67%	N
97	*L. vesuvius*	*L. miqueliana*	5	5	100%	N
98	*L. miqueliana*	*L. wilfordii*	6	0	0%	-
99	*L. wilfordii*	*L. miqueliana*	20	12	60%	N
100	*L. miqueliana*	*L. xhaageana* mixed hybrids	8	4	50%	N
101	*L. xhaageana* mixed hybrids	*L. miqueliana*	10	4	40%	N
102	*L. miqueliana*	*L. xhaageana* 'Lumina Salmon'	1	0	0%	N
103	*L. xhaageana* 'Lumina Salmon'	*L. miqueliana*	4	3	75%	N
104	*L. miqueliana*	*L. xarkwrightii*	2	2	100%	N
105	*L. miqueliana*	*S. armeria*	2	0	0%	-
106	*S. armeria*	*L. miqueliana*	7	0	0%	-
107	*L. miqueliana*	*S. pendula*	4	0	0%	-
108	*S. pendula*	*L. miqueliana*	5	0	0%	-
109	*L. miqueliana*	*S. plankii*	5	0	0%	-
110	*S. plankii*	*L. miqueliana*	3	3	100%	N
111	*L. vesuvius*	*L. flos-cuculi*	7	1	14%	N

112	*L. vesuvius*	*L. wilfordii*	3	2	67%	N
113	*L. wilfordii*	*L. vesuvius*	9	5	56%	N
114	*L. vesuvius*	*L. xhaageana* mixed hybrids	5	2	40%	Y
115	*L. xhaageana* mixed hybrids	*L. vesuvius*	8	8	100%	Y
116	*L. vesuvius*	*S.armeria*	7	1	14%	N
117	*S. armeria*	*L. vesuvius*	11	0	0%	-
118	*L. vesuvius*	*S. plankii*	5	0	0%	-
119	*S. plankii*	*L. vesuvius*	6	2	33%	N
120	*L. vesuvius*	*S. pendula*	6	3	50%	N
121	*S. pendula*	*L. vesuvius*	6	0	0%	-
122	*L. wilfordii*	*L. chalcedonica*	7	1	14%	N
123	*L. wilfordii*	*L. chalcedonica* var. *alba*	6	5	83%	N
124	*L. wilfordii*	*L. xhaageana*	6	4	67%	N
125	*L. xhaageana*	*L. wilfordii*	3	2	67%	N
126	*L. wilfordii*	*L. xhaageana* mixed hybrids	8	5	63%	N
127	*L. xhaageana* mixed hybrids	*L. wilfordii*	4	0	0%	-
128	*L. wilfordii*	*S. armeria*	11	2	18%	N
129	*S. armeria*	*L. wilfordii*	1	0	0%	-
130	*L. wilfordii*	*S. pendula*	8	3	38%	N

131	*S. pendula*	*L. wilfordii*	5	0	0%	-
132	*L. wilfordii*	*S. plankii*	5	0	0%	-
133	*S. plankii*	*L. wilfordii*	5	0	0%	-
134	*L. wilfordii*	*L. xhaageana* 'Lumina Salmon'	1	0	0%	-
135	*L. xhaageana*	*L. chalcedonica* 'Carnea'	2	2	100%	N
136	*L. xhaageana*	*L. chalcedonica* 'White'	2	2	100%	N
137	*L. xhaageana*	*L. cognata*	8	6	75%	Y
138	*L. xhaageana*	*L. flos-cuculi*	1	0	0%	-
139	*L. xhaageana*	*L. miqueliana*	7	5	71%	N
140	*L. xhaageana*	*S. armeria*	6	1	17%	N
141	*S.armeria*	*L. xhaageana*	8	0	0%	-
142	*L. xhaageana*	*S. pendula*	6	5	83%	N
143	*S. pendula*	*L. xhaageana*	5	0	0%	-
144	*L. xhaageana* mixed hybrids	*L. chalcedonica* 'Rauhreif'	1	1	100%	N
145	*L. xhaageana* mixed hybrids	*L. chalcedonica* 'Burning Love'	1	1	100%	N
146	*L. xhaageana* mixed hybrids	*S. plankii*	1	0	0%	-
147	*L. xhaageana* mixed hybrids	*S. pendula*	5	0	0%	-
148	*L. xhaageana* mixed hybrids	*S. acaulis* ssp. *acaulescens*	2	0	0%	-
149	*L. xhaageana* mixed hybrids	*S. armeria*	14	0	0%	-

150	*S. armeria*	*L. xhaageana* mixed hybrids	19	0	0%	-
151	*L. xhaageana* ‘Molten Lava’	*L. chalcedonica* 'White'	2	2	100%	N
152	*L. xhaageana* ‘Molten Lava’	*L. chalcedonica* var. *alba*	1	1	100%	N
153	*L. xhaageana* ‘Molten Lava’	*L. flos-cuculi*	2	0	0%	-
154	*L. xhaageana* ‘Molten Lava’	*L. flos-cuculi* 'White Robin'	1	1	100%	N
155	*L. xhaageana* ‘Molten Lava’	*L. miqueliana*	5	5	100%	Y
156	*L. miqueliana*	*L. xhaageana* ‘Molten Lava’	4	4	100%	N
157	*L. xhaageana* ‘Molten Lava’	*S. pendula*	3	3	100%	N
158	*L. xhaageana* ‘Molten Lava’	*L. xhaageana* mixed hybrids	1	1	100%	Y
159	*L. xhaageana* 'Lumina Orange'	*L. chalcedonica* 'Carnea'	1	1	100%	N
160	*L. xhaageana* 'Lumina Orange'	*L. cognata*	1	1	100%	Y
161	*L. xhaageana* 'Lumina Orange'	*L. miqueliana*	2	1	50%	N
162	*L. xarkwrightii* 'Orange Genome'	*L. chalcedonica* 'Rauhreif'	1	0	0%	-
163	*L. xarkwrightii* 'Orange Genome'	*L. miqueliana*	5	2	40%	N
164	*L. miqueliana*	*L. xarkwrightii* 'Orange Genome'	1	0	0%	-
165	*L. xarkwrightii* 'Orange Genome'	*L. cognata*	1	0	0%	-
166	*L. xarkwrightii* 'Orange Genome'	*S. armeria*	3	0	0%	-
167	*L. xarkwrightii* 'Vesuvius'	*L. cognata*	4	4	100%	Y
168	*L. xhaageana* 'Lumina Brozeleaf Red'	*L. cognata*	1	1	100%	Y

169	*S. armeria*	*L. chalcedonica* 'Carnea'	6	0	0%	-
170	*S. armeria*	*L. flos-cuculi*	2	0	0%	-
171	*S. pendula*	*L. chalcedonica*	5	0	0%	-
172	*S. pendula*	*L. cognata*	3	0	0%	-
173	*L. cognata*	*S. pendula*	9	5	56%	N
174	*S. pendula*	*S. armeria*	5	0	0%	-
175	*S. pendula*	*S. plankii*	3	0	0%	-
176	*S. plankii*	*S. armeria*	1	0	0%	-
177	*S. delavayi*	*L. xarkwrightii*	1	0	0%	-

Table B.2 Hybridizations made with mutant *Lychnis* plants.

Maternal parent[z]	Pollen source[z]	Crossing #	Successful crossing #	Success rate
L. vesuvius	*L. chalcedonica* 0.1C	2	2	100%
L. xhaageana mixed hybrids	*L. chalcedonica* 0.1 C	1	0	0%
L. chalcedonica 1C	*L. cognata*	3	2	67%
L. chalcedonica 1C	*L. xhaageana*	2	1	50%
L. chalcedonica 1C	*L. vesuvius*	1	0	0%
L. cognata	*L. chalcedonica* 1C	1	0	0%
L. vesuvius	*L. chalcedonica* 1C	4	4	100%
L. chalcedonica white	*L. chalcedonica* 1C	8	7	88%
L. chalcedonica alba	*L.chalcedonica* 1C	4	1	25%
L. xhaageana mixed hybrids	*L. chalcedonica* 1C	1	1	100%
L. chalcedonica E	*L. chalcedonica*	1	1	100%
L. chalcedonica E	*L. cognata*	1	0	0%
L. chalcedonica E	*L. miqueliana*	2	0	0%
L. chalcedonica E	*L. xhaageana*	3	0	0%
L. chalcedonica E	*L. vesuvius*	4	0	0%

L. chalcedonica 0.1+E	*L. chalcedonica* var. *alba*	2	1	50%
L. chalcedonica 0.1+E	*L. chalcedonica*	9	3	33%
L. chalcedonica 0.1+E	*L. chalcedonica* ‘White’	2	1	50%
L. chalcedonica 0.1+E	*L. chalcedonica* 'Rauhreif'	2	2	100%
L. chalcedonica 0.1+E	*L. miqueliana*	4	1	25%
L. chalcedonica 0.1+E	*L. xhaageana* ‘Molten Lava’	1	0	0%
L. chalcedonica 0.1+E	*L. cognata*	9	4	44%
L. chalcedonica 0.1+E	*L. wilfordii*	1	0	0%
L. chalcedonica 0.1+E	*L. xhaageana*	4	2	50%
L. chalcedonica 0.1+E	*L. xhaageana* mixed hybrids	11	4	36%
L. chalcedonica 0.1+E	*L. vesuvius*	10	5	50%
L. chalcedonica 0.1+E	*S. armeria*	3	0	0%
L. chalcedonica 0.1+E	*S. pendula*	5	2	40%
L. chalcedonica 0.1+E	*S. plankii*	2	0	0%
S. plankii	*L. chalcedonica* 0.1+E	1	0	0%
L. chalcedonica ‘White’	*L. chalcedonica* 0.1+E	1	1	100%
L. chalcedonica 1+E	*L. chalcedonica*	2	0	0%
L. chalcedonica 1+E	*L. chalcedonica* ‘White’	4	1	25%
L. chalcedonica 1+E	*L. cognata*	19	5	26%

L. chalcedonica 1+E	*L. miqueliana*	5	1	20%
L. chalcedonica 1+E	*L. wilfordii*	2	0	0%
L. chalcedonica 1+E	*L. xhaageana*	11	2	18%
L. chalcedonica 1+E	*L. xhaageana* 'Molten Lava'	2	0	0%
L. chalcedonica 1+E	*L. xhaageana* mixed hybrids	10	0	0%
L. chalcedonica 1+E	*L. vesuvius*	8	0	0%
L. chalcedonica 1+E	*S. armeria*	4	0	0%
L. chalcedonica 1+E	*S.pendula*	1	0	0%
L. chalcedonica 1+E	*L. xhaageana* 'Molten Lava'	1	0	0%
L. flos-cuculi E	*L. cognata*	2	0	0%
L. flos-cuculi E	*L. xhaageana* mixed hybrids	1	0	0%
L. flos-cuculi E	*L. xhaageana* 'Lumina Orange'	1	0	0%
L. flos-cuculi E	*L. miqueliana*	9	0	0%
L. flos-cuculi 0.1+E	*L. chalcedonica* var. *alba*	3	0	0%
L. flos-cuculi 0.1+E	*L. xhaageana* 'Molten Lava'	3	0	0%
L. flos-cuculi 0.1+E	*L. vesuvius*	4	0	0%
L. flos-cuculi 1+E	*L. chalcedonica* 'Burning Love'	2	0	0%
L. flos-cuculi 1+E	*L. miqueliana*	1	0	0%
L. flos-cuculi 1+E	*L. xhaageana*	5	0	0%

L. flos-cuculi 1+E	*L. xhaageana* mixed hybrids	5	0	0%
L. flos-cuculi 1+E	*L. wilfordii*	2	0	0%
L. flos-cuculi 1+E	*L. cognata*	5	0	0%

[z]All chemical mutagen treated species with the symbols 0.1C, 1 C, E, 0.1 +E, or 1+E represent soaking seeds in 0.1 % caffeine, 1% caffeine, 0.6% EMS, 0.1% caffeine plus 0.6% EMS and 1% caffeine plus 0.6% EMS for 24 hours, respectively.

Table B.3 Selfing of *Lychnis* plants for different purposes.

Maternal parent[z]	Crossing #	Selfing purpose
L. cognata	21	For double flowers
L.flos-cuculi	4	For vivipary
L. xhaageana	1	For white flowers
L. xhaageana mixed hybrids	16	Color selection
L.miqueliana	13	For seeds
L.chalcedonica 0.1 C	4	Mutants
L.chalcedonica 1 C	1	Mutants
L.chalcedonica 0.1+E	9	Mutants
L.chalcedonica 1+E	16	Mutants
L.chalcedonica 1 C	1	Mutants
L.chalcedonica E	3	Mutants
L.flos-cuculi 1+E	1	Mutants

[z] Chemical mutagen treated species with the symbols 0.1C, 1 C, E, 0.1 +E, or 1+E represent soaking seeds in 0.1 % caffeine, 1% caffeine, 0.6% EMS, 0.1% caffeine plus 0.6% EMS and 1% caffeine plus 0.6% EMS for 24 hours, respectively.

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Scope and Method of Study: This study focused on conventional breeding in the genus *Lychnis* L. involving two different breeding methods and asexual propagation, which is an essential horticultural technique to facilitate ornamentals reproduction including hybrids. Intraspecific, interspecific, and intergeneric hybridizations and chemical mutagenesis were investigated both for generating desirable ornamental traits and methodology research. Also stem and leaf cuttings were attempted to initial establishment of asexual propagation procedures for *Lychnis*.

Findings and Conclusions: Several desirable hybrids and many mutants were selected. Results revealed that hybrid seed morphology indicates hybridization success, and cytoplasmic inheritance reflected by some *Lychnis* traits. The sensitivity to different mutagens was variable among selected species. Caffeine showed an effect on *Lychnis*, whereas the effectiveness as a breeding mutagen calls for further investigation. Best media and hormone combinations for rooting were determined for two experimental *Lychnis* species, repectively, for the first time.

ADVISER'S APPROVAL: Dr. Bruce L. Dunn