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Variability analysis and evaluation for major cut flower traits of F_1 hybrids in *Lilium brownii* var. *colchesteri*

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Abstract. The characteristics of *Lilium brownii* var. *colchesteri* are not found in other lily species. This study analyzed twelve genotypes (F_1 hybrids) of *L. brownii* var. *colchesteri* for nine important cut flower characteristics. As part of pre-breeding, the variability analysis task for the studied traits was carried out in a randomized complete block design with three replications. The analysis of variance (ANOVA) analyses demonstrated significant variability among the genotypes for the studied cut-flower traits. The higher values of the phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), coupled with higher heritability, along with the genetic advance expressed as a percentage mean (GAM), revealed that plant height, leaf length, leaf burn, and flower diameter had demonstrated the prevalence of the additive type of gene action. The remaining cut flower traits, especially leaf width, number of flowers, length of outer tepal, the width of outer tepal, and days to flowering, proved to have a prevalence of the non-additive type of gene action. The mean performance of the cut flower traits provides information for effective progeny selection after hybridization. The performance of Ryunghoehyeon had been found to be outstanding for plant height, flower diameter, length of the outer tepal, width of the outer tepal, days to flowering, and the number of flowers.

Keywords: *Lilium brownii* var. *colchesteri, F_1 hybrids, cut flower traits, heritability, and genetic advance as percent of mean*

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1. Introduction

Lilies are one of the perennial floricultural crops, and the genus *Lilium* represents most of the lilies, which comprise 110 species, seven sections, and more than 10,000 documented cultivars worldwide, with thousands of cultivars recorded each year. (McRae, 1998; Mathews, 2007; Bakhshaie et al., 2016). They have spread worldwide, primarily confined to the northern hemisphere and mainly found in Asia, North America, and Europe. East coast areas of Asia, particularly in China, Japan, and Korea, are rich habitats for many wild species (Hai et al., 2012a; Dhiman et al., 2018). Following the coastal regions of southwest and north China, which are considered the primary centers of wild lily diversity with 59 species of wild lilies (De Jong, 1974), Korea is another important and rich wild lily habitat with 13 wild lilies, including 11 wild species and two varieties (Lighty, 1969; Lee et al., 2011; Lucidos, 2013). They represented three classes: the Sinomartagon section (7 species), the Martagon section (3 species), and the Daurolirion section (1 species) (Lee, 2003; Kim, 2008).

Lilium brownii var. *colchesteri* comes under Lecucolirion based on the flower shapes (Wilson, 1925) and is classified under Archelirion based on the seed germination manner, leaf arrangement, bulb scale, shape, and habit, seed characters, and flower shapes (Comber, 1949). But recent studies have proved that along with its closely related species, i.e., *L. formosannum* Wallace and *L. longiflorum* Thunb., it has come under the Leucolirion section (Nishikawa et al., 2001). *Lilium brownii* F.E. Brown var. *colchesteri* Wilson is supposed to have originated and been found in its natural wild forms in almost all parts of central China and spread from the northern to the southern provinces of China (Shimizu, 1971; Liang, 1980; McRae, 1998; Okubo et al., 2012), and from its origins it spread over Japan, Korea, and Europe (Okubo, 2006; Okubo et al., 2012). Moreover, there has been evidence of cultivation in Japan since more than 400 years ago (Okubo, 2006). According to Okubo et al. (2012), it is synonymously known as *L. brownii* var. *viridulum*, Baker. Likewise, Hakata Lily commonly included *L. brownii* var. *colchesteri*, excluding *L. brownii*. In comparison to other Lily species, the distinctive *L. brownii* var. *colchesteri* displayed distinct ornamental characteristics, such as changing flower color from yellowish cream during anthesis to white after one day. It possesses an amazing sweet fragrance with graceful petals and anthers (Hai et al., 2012a; Hai et al., 2012b). Moreover, *L. brownii* var. *colchesteri* demonstrated many more traits, which are prerequisites for both cut and pot flower traits. Though having these peculiar ornamental traits, *L. brownii* var. *colchesteri* has demonstrated that virus resistance is feeble as it is susceptible to different types of viruses, viz., LSV (Lily Symptomless Virus), LMoV (Lily Mottle Virus), CMV (Cucumber Mosaic Virus), Fusarium wilt, and soil mites. In this context, the objective of breeding in L. brownii var. colchesteri is to produce virus-free hybrids and new cultivars with other desirable cut and pot flower traits (Masuda et al., 2011).

Many breeding efforts are made to create inter-specific hybrids between *L. brownii* var. *colchesteri* and its closely related species, *L. formolongi*, *L. longiflorum*, and *L. formosanum*, to make a new cultivar with sweet-smelling flowers that change color (Saruwatari et al., 2007; Saruwatari et al., 2008; Okubo et al., 2012; Hai et al., 2012a; Hai et al., 2012b). Since *Lilium brownii* var. *colchesteri* is self-incompatible (Shimizu, 1971), *L. brownii* sets seeds when crossed with var. *colchesteri* or var. *australe*. It might be possible to obtain seeds by crossing different landraces or varieties (McRae, 1998). In this experiment, we evaluated twelve F_1 hybrids of *L. brownii* var. *colchesteri* for major cut flower traits as pre-breeding tasks for hybrid screening and recommendations for future breeding.

2. Materials and Methods

2.1. Plant material and sites

Twelve F_1 hybrid genotypes (Table 1) employed in this experiment were provided by the Bio-herb Research Institute, Floricultural Breeding Laboratory, under the Department of Horticulture, College of Agriculture and Life Sciences, Kangwon National University (KNU), South Korea. The experiment was carried out in the central experimental field of KNU at 37° 52' N latitude and 127° 44´ E in 2020.

2.2. Field experimental design and raising plant saplings

To carry out this experiment, 12 F_1 hybrid genotypes of commercial-sized bulbs aged 2-3 years were obtained as standing crops at KNU's central experimental farm. For the execution of this experiment, the whole plot was considered a block consisting of 20 beds, and each bed measured 30 m long and 1.2 m wide. For the ease of carrying out intercultural operations, viz., weeding, watering, application of herbicide, insecticide, and fertilizer, as well as morphological observation of crops, the space between the beds was managed at 0.6-0.75 m. Furthermore, the bulbs of all genotypes were transplanted, maintaining plant-to-plant and row-to-row equidistance of 12.5 cm, following the standard set by the local lily growers and farmers. All of the bulbs were planted and grew using the randomized complete block design (RCBD) with three replications.

2.3. Morphological observation and data collection

During the main season of flowering in the Chuncheon condition, starting from the third week of June to the first week of August, the morphological observation of major cut flower traits (Tables 2 and 3) was carried out by taking the three representative plant saplings from each replication. Following the rules of the Lily Register Checklists (Mathews, 2007) and the general method of measuring traits used by Rai et al. (2018), nine cut flower traits were measured for each genotype by taking samples from three plants from each replication, for a total of nine plants for each genotype.

2.4. Statistical analysis and interpretation of data

The raw data obtained through morphological observation of all genotypes of nine studied cut flower traits were prepared using MS-Excel 2013. The replication-wise mean data of each genotype were employed for the further analysis of variance (ANOVA) and other variability analysis parameters, viz., phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability, genetic advance, and percent mean for major cut flower traits. The ANOVA analyses, F test, and other variance components were analyzed using the TNAUSTAT statistical package for the general plant breeding for the randomized complete block design (RCBD) with three replications. Duncan's multiple range test (DMRT) was estimated at the

5% significance level for separating the estimated means of all genotypes using the same designs, using the TNAUSTAT statistical package's DMRT comparison tools (Manivannan, 2014). Likewise, all the variance components and their variance coefficients (namely, phenotypic, genotypic, and environmental) were estimated according to the formula and detailed methods provided by Burton and Devane (1953). Furthermore, at last, broad sense heritability (H^2), genetic advance (GA), and genetic advance as a percent of the mean (GAM) were estimated based on the formula provided by Allard (1960).

Table 1. Name of F_1 hybrids, their special characteristics, and resource center for *L. brownii* var. *colchesteri* genotypes (F_1 hybrids)

S.N.	**Designation of genotypes**	**Special remarks**	**Resource**
1	Ryung × Hwa	Short plant height and intermediate flowering habit	Bio-herb Research Institute/Floricultural Breeding Laboratory, KNU
2	Hwa × Kuiju	Short plant height and intermediate flowering habit	Bio-herb Research Institute/Floricultural Breeding Laboratory, KNU
3	Ryung OP	Short plant height, intermediate flowering habit, and botrytis infestation of less than 20%	Bio-herb Research Institute/Floricultural Breeding Laboratory, KNU
4	(Hwa × Kui) × Inhwa	Short plant height, early flowering, and botrytis infestation of less than 5%	Bio-herb Research Institute/Floricultural Breeding Laboratory, KNU
5	Dangdong × Jindo	Middle plant height and flowering habit	Bio-herb Research Institute/Floricultural Breeding Laboratory, KNU
6	No.6	Plant height and flowering time are intermediate, and botrytis infestation is less than 10%	Bio-herb Research Institute/Floricultural Breeding Laboratory, KNU
7	Hwa × Baeksu	Short plant height, intermediate flowering habit, and botrytis infestation of less than 10%	Bio-herb Research Institute/Floricultural Breeding Laboratory, KNU
8	Inhwa × Kuiju	Short plant height, early flowering habit, and botrytis infestation up to 20%	Bio-herb Research Institute/Floricultural Breeding Laboratory, KNU
9	Ryunghoehyeon	Tall plant height, early flowering habit, and botrytis infestation of less than 10%	Bio-herb Research Institute/Floricultural Breeding Laboratory, KNU
10	(Hyeon × Baek) × Hwa	Short plant height, early flowering habit, and botrytis infestation of less than 5%	Bio-herb Research Institute/Floricultural Breeding Laboratory, KNU
11	IT 282748	Tall plant height, mid-flowering habit, and botrytis infestation of less than 5%	Bio-herb Research Institute/Floricultural Breeding Laboratory, KNU
12	IT282736	Short to medium plant height, late flowering habit, and less than 5% botrytis infestation	Bio-herb Research Institute/Floricultural Breeding Laboratory, KNU

Note: OP = open-pollinated, KNU = Kangwon National University, SN = serial number

3. Results and Discussion

3.1. The analysis of variance (ANOVA) and its components for major cut flower traits

The estimated analysis of variance (ANOVA) (Table 2) revealed that all the cut flower traits studied, i.e., plant height, leaf length, leaf width, leaf burn, flower diameter, the number of flowers, length of the outer tepal, the width of the outer tepal, and days to flowering, significantly varied among the genotypes. It denotes that all twelve F_1 hybrids have the potential for the varied performance of the nine cut flower traits.

Table 2. The ANOVA and genetic parameters for the most important cut flower traits in *L. brownii* var. *colchesteri* genotypes (F_1 hybrids)

Traits / Parameter	**G. Mean ± SEM**	**LSD (5%)**	**CV (%)**	**F test**	**PCV (%)**	**GCV (%)**	**ECV (%)**	**H^2 (%)**	**GA**	**GAM (%)**
1. Plant height	60.06±1.10	19.31	1.93	**	16.26	16.14	1.93	98.59	14.14	33.02
2. Leaf length	9.92±0.15	2.60	3.60	**	12.63	12.11	3.60	91.86	4.06	23.91
3. Leaf width	1.72±0.02	0.42	3.91	**	9.11	8.22	3.90	81.57	0.68	15.30
4. Leaf burn	31.32±1.16	20.43	9.66	**	38.65	37.42	9.66	93.75	35.05	74.64
5. Flower diameter	44.07±0.56	9.76	2.14	**	12.63	12.44	2.14	97.14	11.30	25.27
6. No. of flowers	2.57±0.07	1.14	11.42	**	15.67	10.73	11.43	46.85	1.70	15.12
7. Length of OT	14.82±0.11	1.88	1.92	**	6.45	6.16	1.92	91.12	3.21	12.11
8. Width of OT	3.79±0.04	0.74	2.72	**	9.42	9.02	2.72	91.68	1.17	17.79
9. Days to flowering	82.3±0.46	8.00	0.65	**	5.34	5.30	0.65	98.54	6.47	10.84

Note: OT = outer tepal, SEM = standard error mean, LSD = least significant difference, CV = coefficient of variation, PCV = phenotypic coefficient of variation, GCV = genotypic coefficient of variation, ECV = environmental coefficient of variation, H^2 = heritability, GA = genetic advance, GAM = genetic advance as percentage of mean, No = number

For all nine cut flower traits, the phenotypic components of variance (PCV) were found to be bigger than the genotypic components of variance (GCV) in Table 2. This means that the environment has a bigger effect on how these cut flower traits are expressed. The estimated PCV and GCV ranged from the lowest 5.34 and 5.30 (for days to flowering) to the highest 38.65 and 37.42 (for leaf burn). Based on estimated PCV and GCV (Table 2), out of the nine studied cut flower traits, leaf burn possessed high PCV and GCV values.

In contrast, plant height, leaf length, flower diameter, and the number of flowers demonstrated medium PCV and GCV values. The remaining cut flower traits, i.e., leaf width, length of outer tepal, width of outer tepal, and days to flowering, had been recorded with lower PCV and GCV values (Deshmukh et al., 1986). The leaf burn had demonstrated enormous scope for selection and hybridization (Khan et al., 2009), as it had indicated the presence of exploitable genetic variability (Yadav et al., 2009). Furthermore, acceptable improvements could be obtained through selection and hybridization for the plant height, leaf length, flower diameter, and number of flowers. The remaining cut flower traits, namely leaf width, length of outer tepal, width of outer tepal, and days to flowering, revealed a low level of heritable genetic material, indicating that non-additive genetic action was important for these cut flower traits.

3.2. Estimated heritability of the major cut flower traits

Heritability is one of the insights that breeders use to make selection and hybridization decisions in any breeding scheme. Heritability provides an idea of the genetic control for the expression of particular traits and the reliability of the phenotype for the prediction of breeding value (Ndukauba et al., 2015). The details of the estimated heritability for the nine cut-flower traits are given in Table 2. The heritability estimates ranged from the lowest, 46.85, for the number of flowers, to the highest, 98.59, for plant height. The estimated heritability demonstrated very high heritability for most traits, i.e., 98.89 for plant height, 98.54 for days to flowering, 97.14 for flower diameter, 93.75 for leaf burn, 91.86 for leaf length, 91.68 for width of the outer tepal, 91.12 for length of the outer tepal, and 81.57 for leaf width, besides moderate heritability, i.e., 46.85 for the number of flowers (Singh, 2001). Because of the very high estimated heritability, there should be little environmental influence on the observed variation for these traits. Selection is a good way to make improvements that can be used in the future to breed for these traits.

3.3. The estimated genetic advance (GA) and genetic advance as percent of the mean (GAM) for major cut flower traits

It is one of the most important insights for evaluating germplasm as a pre-breeding task after the ANOVA and its components of variance and heritability estimates. Both the genetic advance (GA) and genetic advance as percent of the mean (GAM) are given in Table 2. The estimated GAM ranged from 10.84 (the lowest for days to flowering) to 74.64 (the highest for leaf burn). Out of nine cut flower traits, plant height, leaf length, leaf burn, and flower diameter possessed high GAM. In contrast, the remaining cut flower traits, viz., leaf width, number of the flower, length of the outer tepal, width of the outer tepal, and days to flowering, possessed moderate GAM. It has clear evidence that cut flower traits like plant height, leaf length, leaf burn, and flower diameter have high heritability as well as a high heritability estimate, implying effective progeny selection after hybridization for these traits because we discovered the prevalence of additive gene action for these traits (Tazeen et al., 2007; Ndukauba et al., 2015). The combined study of heritability and GAM is more informative than heritability alone (Ullah et al., 2012; Gebregergs & Mekbib, 2020).

3.4. The estimated mean performances for the major nine cut flower traits

The evaluation of the mean performance of the studied cut flower traits is another important criterion for evaluating the genotypes for the particular cut flower traits since all the studied genotypes demonstrated significantly varied (Table 2, F test) cut flower traits. The mean performance of all genotypes for nine cut flower traits is given in Table 3. The F_1 hybrids Ryunghoehyeon and IT 282748 were the tallest, while the F_1 hybrids Hwa × Kuiju and Inhwa × Kuiju were found to be the shortest. The longest leaves were found for the hybrids (Hwa × Kui) × Inhwa, and (Hyeon × Baek) × Hwa. While the shortest leaves were found for the hybrids Dangdong × Jindo, and No. 6. Furthermore, the broadest leaves were found for the hybrid (Hyeon × Baek) × Hwa, while the narrowest leaves were found for Ryung × Hwa and IT282736. Leaf burn is another important cut flower trait. Usually, we prefer genotypes with a lower amount of leaf burn. In this context, the lowest percentage of leaf burn was found for Hwa × Baeksu and IT 282748, while the highest percentage of leaf burn was found for the genotypes, Ryung × Hwa, and IT 282736. Another important cut flower trait is flower diameter; the biggest flowers were measured for No. 6, while the smallest flower was measured for IT282736. Likewise, the highest number of flowers was counted for No. 6, and the lowest number of flowers was found for Inhwa × Kuiju. Outer tepal length and width are other essential criteria from the cut flower's point of view. The longest outer tepal was found in the genotypes of No. 6 and Ryunghoehyeon, while the shortest outer tepal was measured for the genotypes of Hwa × Kuiju and (Hyeon × Baek) × Hwa. On the other hand, the broadest outer tepal was recorded for Ryunghoehyeon, while the narrowest outer tepal was measured for Hwa × Kuiju, (Hwa × Kui) × Inhwa, Dangdong × Jindo, and Hwa × Baeksu. The last cut flower trait of our present study but most important from the cut flower point of view is none other than days to flowering; the early flowering habit was found in the genotypes of (Hwa × Kui) × Inhwa, Ryunghoehyeon, and (Hyeon × Baek) × Hwa, while the late flowering genotype was recorded for IT 282748. The mean performance (Table 3) revealed that the hybrid, Ryunghoehyeon, had outstanding performance for plant height, flower diameter, length of the outer tepal, width of the outer tepal, days to flowering, and the number of flowers (3.10). Rai et al. (2018) said that these different mean performances for cut flower traits are caused by the different genotypes.

Table 3. The mean performance for major cut flower traits in *L. brownii* var. *colchesteri* genotypes (F_1 hybrids)

Traits / Genotypes	**1. Plant height**	**2. Leaf length**	**3. Leaf width**	**4. Leaf burn**	**5. Flower diameter**	**6. No.of flowers**	**7. Length of OT**	**8. Width of OT**	**9. Days to flowering**
1. Ryung × Hwa	59.53^{c}	9.23^{cd}	1.57^{df}	49.10^{a}	47.20^{c}	2.67^{bc}	15.4^{b}	4.0^{b}	81.4^{e}
2. Hwa × Kuiju	51.43^{f}	9.37^{c}	1.63^{de}	32.50^{cd}	40.87^{gh}	2.43^{cd}	13.5^{e}	3.5^{e}	83.8^{bc}
3. Ryung OP	53.77^{e}	9.27^{cd}	1.60^{de}	43.20^{b}	43.05^{ef}	2.30^{cd}	14.7^{c}	3.7^{cd}	82.0^{e}
4. (Hwa × Kui) × Inhwa	56.57^{d}	12.03^{a}	1.70^{cd}	30.60^{de}	44.9^{1d}	2.57^{bc}	14.5^{cd}	3.5^{e}	77.8^{g}
5. Dangdong × Jindo	60.10^{c}	8.60^{d}	1.77^{bc}	33.20^{cd}	50.32^{b}	2.67^{bc}	14.3^{cd}	3.5e	81.8^{e}
6. No.6	63.77^{b}	8.63^{d}	1.87^{b}	36.30^{c}	41.77^{fg}	3.20^{a}	16.0^{a}	4.1^{b}	84.6^{b}
7. Hwa × Baeksu	54.23^{e}	9.87^{c}	1.77^{bc}	15.30^{g}	46.99^{c}	2.33^{cd}	14.7^{c}	3.5^{e}	83.1^{cd}
8. Inhwa × Kuiju	49.87^{f}	10.70^{b}	1.70^{cd}	24.20^{f}	42.05^{ef}	2.10^{ce}	13.9^{de}	3.6^{d}	78.9^{f}
9. Ryunghoehyeon	79.1^{a}	9.33^{c}	1.80^{bc}	26.40^{ef}	54.85^{a}	3.10^{ab}	16.4^{a}	4.6^{a}	77.0^{g}
10. (Hyeon × Baek) × Hwa	53.80^{e}	12.27^{a}	2.07^{a}	22.30^{f}	43.55^{de}	2.43^{cd}	13.6^{e}	3.6^{cd}	77.8^{g}
11. IT 282748	79.00^{a}	10.50^{b}	1.60^{de}	13.20^{g}	40.32^{h}	2.33^{cd}	15.4^{b}	3.6^{cd}	87.2^{a}
12. IT282736	59.53^{c}	9.23^{cd}	1.57^{df}	49.10^{a}	32.90^{i}	2.67^{bc}	15.4^{b}	3.8^{c}	82.3^{de}
G. Mean ± SEM	**60.06±1.10**	**9.92±0.15**	**1.72±0.02**	**31.3±1.16**	**44.07±0.56**	**2.57±0.07**	**14.8±0.11**	**3.79±0.04**	**82.3±0.46**

Note: OP = open pollinated; OT = outer tepal

4. Conclusions

In this study, we have analyzed twelve genotypes (F_1 hybrids) of *L. brownii var. colchesteri* for nine commercially important major cut flower traits, viz. plant height, leaf length, leaf width, the number of leaves burned, the number of flowers, flower diameter, the length of the outer tepal, the width of the outer tepal, and days to flowering. The statistical analysis of highly significant variability among the genotypes for all the studied traits thereby provides validity for evaluating genotypes as pre-breeding tasks. Plant height, leaf length, leaf burn, and flower diameter have higher PCV and GCV values and higher heritability estimates, which confirm the additive gene action prevalence for this study and show that progeny selection after hybridization is the best way to breed these traits. On the other hand, leaf width, number of flowers, length of the outer tepal, width of the outer tepal, and days to flowering have lower PCV and GCV values and lower heritability estimates.

Conflicts of interest. The authors said that none of them have a conflict of interest when it comes to this article.

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