IDENTIFICATION OF SUPERIOR THREE WAY-CROSS F_1 S, ITS LINE $\times$ TESTER HYBRIDS AND DONORS FOR MAJOR QUANTITATIVE TRAITS IN *Lilium* $\times$ *formolongi*

Rameshwar Rai¹, Amarsanaa Badarch¹, Jong-Hwa Kim^{1, 2*}

¹Department of Horticulture, Kangwon National University, Chuncheon 24341, Korea

²Oriental Bio-herb Research Institute, Kangwon National University, Chuncheon 24341, Korea

Received – March 01, 2020; Revision – April 17, 2020; Accepted – April 24, 2020

Available Online – April 25, 2020

DOI: [http://dx.doi.org/10.18006/2020.8\(2\).157.165](http://dx.doi.org/10.18006/2020.8(2).157.165)

KEYWORDS

Three way-cross F_1 s

L $\times$ T hybrids

ANOVA for L $\times$ T analysis

Randomized Complete Block Design (RCBD)

Quantitative traits

Lilium $\times$ *formolongi*

ABSTRACT

This experiment was carried out to identify superior parents [three way-cross F_1 s and donors (cultivars/breeding line)], and their Line $\times$ Tester hybrids (hereafter L $\times$ T hybrids) for major quantitative traits in *Lilium* $\times$ *formolongi*. The experiment was designed in line $\times$ tester mating design taking 5 three way-cross F_1 s as lines, 3CVs/breeding lines as testers and their 15 L $\times$ T hybrids in a Randomized Complete Block Design (RCBD) with 3 replications. The analysis of variance (ANOVA) for L $\times$ T analysis revealed a highly significant difference for all the studied quantitative traits revealing the presence of genetic variability among the genotypes under investigation. The estimated GCA effects revealed three way-cross F_1 s; (Stu $\times$ W) $\times$ AF1-6 (L2) and (AF $\times$ Ad) $\times$ HU-3(L1) found to be superior as they possessed significant GCA effects for 7 and 4 quantitative traits respectively. Likewise, Julius-19 (T1) and 12-1-2(T3) can be used as effective donors as both of them possessed significant GCA effects for 6 quantitative traits. The L $\times$ T hybrids, [57-7 (Aug $\times$ AugE) $\times$ BT] $\times$ 12-1-2 i.e. L4 $\times$ T3 and [60.1 (AF $\times$ Ad) $\times$ Gel] $\times$ Julius-19 i.e. L5 $\times$ T1 has possessed significant SCA effects for 5 traits and [(Stu $\times$ W) $\times$ AF1-6] $\times$ Julius-19 i.e. L2 $\times$ T1, [(Stu $\times$ W) $\times$ AF1-6] $\times$ WT-5 i.e. L2 $\times$ T2 and [(Stu $\times$ W) $\times$ AugE-2] $\times$ WT-5 i.e. L3 $\times$ T2 had revealed significant SCA effects for 4 traits. These hybrids can be considered as promising hybrids. The estimation of gene action divulged that bud length and the attitude of the floral axis shown the prevailing of additive gene action while the rest of the traits had predominantly exhibited non-additive gene action.

* Corresponding author

E-mail: jonghwa@kangwon.ac.kr, rairsansk@gmail.com (Jong-Hwa Kim)

ORCID: <https://orcid.org/0000-0002-9300-6465>

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI]
(<http://www.horizonpublisherindia.in/>).
All rights reserved.

All the articles published by [Journal of Experimental Biology and Agricultural Sciences](#) are licensed under a [Creative Commons Attribution-NonCommercial 4.0 International License](#) Based on a work at www.jebas.org.

1 Introduction

Lily (*Lilium* L., $2n=2x=24$) is one of the most important ornamental bulbous crops which belongs to the family Liliaceae, includes 110 species (McRae, 1998), 7 sections and more than 10000 documented cultivars (Mathews, 2007; Bakhshaie et al., 2016) which increases year by year. It is a perennial herb that possesses scaly bulb, unbranched stem, and smooth/pubescent, usually bright green, sometimes tinged purple or brown and generally covered with leaves. The native *Lilium* species are spread over the Northern Hemisphere and centered mainly in Asia, North America, and Europe (Van Tuyl & Arens, 2011). Lilies are economically most important bulbous crops mostly used for cut flower, potted plants, some species used as edible bulbs or medicinal use in Eastern Asia (Bakhshaie et al., 2016). According to Lucidos et al. (2013) due to its multi aspects viz. diversity of flower color, flower shape, long and multi flowering stem and having long post-harvest shelf life; lilies are highly demanded for its cut flowers in recent international flower trade.

The *Lilium* $\times$ *formolongi* hort is an interspecific hybrid obtained through crossing of two *Lilium* species i.e. *L. formosanum* and *L. longiflorum*. It is popular in East Asia (especially Korea, China and Japan) as commercial cut flower in flower market (Ho et al., 2006; Grassotti & Gimelli, 2011; Rai et al., 2018).

The single cross F_1 s lack population buffering and possess only individual buffering so it was therefore apprehended that single cross may not perform as stable as double cross and three way-cross hybrids (Allard & Bradshaw, 1964). As three way-cross have both populations and individual buffering; three way-cross hybrids are intermediate between single and double cross hybrids concerning uniformity, yield, stability and the relative simplicity of selecting and testing (Weatherspoon, 1970; Schnell, 1975). Keeping this point in mind, current study used three way-cross hybrids as mother lines.

The Line $\times$ Tester mating design is one of the helpful tools available to the breeder for quantitative plant breeding analysis. Basically it is the extension of top cross where only one tester used while more than one tester are used in L $\times$ T mating design. (Nduwumuremyi et al., 2013). It involves hybridization between lines (f) and wide based testers (m) in one to one fashion for generating $f \times m = fm$ hybrids (Sharma, 2006). Furthermore, it provides GCA of both lines and testers and SCA of each cross (Sharma, 2006). At last it is very useful for estimating various types of gene actions important for the expression of quantitative traits (Rashid et al., 2007).

The *Lilium* $\times$ *formolongi* is used for cut flower production mainly as seed propagation, seeds used to sown during December-February (upon the availability of labor) usually flowered during

July-August (Goo et al., 2003; Xuan et al., 2005). As populations derived from the seed propagation are not uniform in plant height, flowering time and flower shape (Roh, 2002) and the stability of novel cut flower traits is a major concern for commercial-scale production (Ho et al., 2006) in this experiment clonal lines (scaled) of parental material was used to maintain the heterozygous genotypes for the seed production of a homogenous population. In this way identification of appropriate mother lines i.e. three way-cross F_1 s and its proper donor (tester) and their promising L $\times$ T hybrids for major quantitative traits are the major concern of this experiment.

2 Materials and Methods

2.1. Plant material, generation of crosses and field experiment layout

The series of experiments plant material preparation, generation of crosses according to Line $\times$ Tester mating design and the final step of experiment were carried out in the backside school field, main gate field and experimental farm of KNU at Chuncheon, Kangwon-Do, South Korea respectively during the season of 2016 to 2018. The seeds of three-way cross F_1 s and donor cultivar and breeding lines were obtained from KNU, Department of Horticulture, Floricultural breeding lab in 2016 winter for the preparation of seedlings. During the summer season of 2016 based on morphological observation of major growth and flowering traits lines of five promising three-way cross F_1 s and three clonal lines of broad-based donor cultivar and breeding, lines have been selected for the purpose to be used as lines and testers respectively. The details of parental materials (Lines and testers) are given in Table 1. In the succeeding year, 15 L $\times$ T hybrids have been prepared to hybridize those 5 three-way cross F_1 s and three donors in the Line $\times$ Tester method. The seedlings of altogether 23 genotypes (5 lines, 3 testers, and 15 L $\times$ T hybrids) has been prepared during the January to April in 2018 following the method as described by Rai et al. (2018) inside plastic house and have been transplanted in the main field in the last week of April. The field experiment has been carried out in RCBD plot design with maintaining 3 replications. The land preparation, fertilizers and insecticides application, as well as other intercultural operations, has been carried out following the Rai et al. (2019).

2.2 Measurement of quantitative traits

The measurement of major quantitative traits has been done during the main flowering season i.e. from last week of June to the first week of August; taking samples of 12 reliable plants from each replication. The generation of crosses preparation procedure and measurement of morphological traits have been adopted from Rai et al. (2018).

Table 1 The list of parental materials (Lines and testers) and special remarks of the traits

S.N.	Genotypes	Source	Remarks
(a) Lines			
L1	(AF×Ad) × HU- 3	KNU, Floricultural breeding lab.	PHT Intermediate to tall, floral axis upward facing
L2	(Stu× W) × AF ₁ -6	KNU, Floricultural breeding lab.	Taller ,floral axis upward facing, strong but late flowering
L3	(Stu× W) × AugE-2	KNU, Floricultural breeding lab.	PHT Intermediate, up directional floral axis, early flowering
L4	57-7(Aug×AugE)×BT	KNU, Floricultural breeding lab.	PHT Intermediate to tall, up directional floral axis.& strong
L5	60.1(AF×Ad)×Gel	KNU, Floricultural breeding lab.	PHT Intermediate, early flowering ,strong and up directional floral axis
(B) Testers			
T1	Julius-19	KNU, Floricultural breeding lab.	Intermediate, very early flowering & up facing floral axis
T2	WT-5	KNU, Floricultural breeding lab.	PHT Intermediate to tall, early flowering & near up floral axis
T3	12-1-2	KNU, Floricultural breeding lab.	Late flowering ,up facing floral axis ,number of flower and thick stem

L1=Line 1.....L5=Line 5, T1=Tester 1.....T3=Tester 3, KNU=Kangwon National University, PHT=Plant height

2.3 Statistical analysis

The preparation of all recorded data of all traits has been done using MS-Excel-2013 and TNAUSTAT statistical package. The ANOVA for L×T combining ability analysis was carried out following the method suggested by Kempthorne (1957). Furthermore, estimation of GCA effects, SCA effects as well the standard errors for testing the significance of GCA and SCA effects, the estimation of components of genetic variance, the proportional contribution of lines, testers and line×tester interactions to the total variance and mean performance for all traits of lines, testers, and L×T hybrids were performed adopting the software of TNAUSTAT statistical packages (Manivannan, 2014). Likewise, based on the overall GCA effects of their involved parents, the ranking of the best specific combiner has been arranged for the particular traits according to the method outlined by Arunachalam & Bandyopadhyay (1979).

3 Results

3.1 The mean performance of studied traits

The performance of all 23 genotypes (5lines, 3testers, and 15 L×T hybrids) for all studied traits have been measured and estimated individually and presented as the group-wise overall mean performance of lines, testers and L×T hybrids (Figure 1). The mean performance of testers demonstrated higher for plant height, stem diameter, number of leaves, leaf length, leaf width, number of flowers, days to flowering (lower number means earlier flowering and taken as positive way) and attitude of the floral axis as compared to the mean performance of lines. That is why testers have selected for the improvement of those respective traits for lines. On the other hand, better performance for floral diameter and

attitude of the floral axis has been observed in L×T hybrids than lines.

3.2 The ANOVA for L×T analysis

The mean sum square of all genotypes for all studied quantitative traits for L×T hybrids (crosses), lines and testers are revealed highly significant. Furthermore, mean sum square of L×T interaction sources of variance has demonstrated that all most traits studied besides bud length and attitude for floral axis were highly significant (Table 2). In this way the ANOVA for L×T analysis indicated significant genetic variability among the genotypes of lines, testers and L×T hybrids for studied traits.

3.3. Gene action and contribution of line, tester, and line×tester interaction

According to the estimated gene action as shown in Table 4; eight studied traits possessed non-additive gene action while rest of the traits viz. bud length and the attitude of the floral axis has demonstrated the prevailing of additive gene action.

As shown in Table 3, the range of contribution (in percentage) of lines found to be highest for the attitude of the floral axis (75.90%) while it is appeared to be lowest (27.03%) for stem diameter. Likewise in case of donors (Testers), the highest contribution appeared for leaf width (47.50%) and lowest for number of leaves (6.24%). In other hand, the maximum contribution of L×T interaction has appeared for stem diameter (43.35%) and the minimum contribution has appeared for the attitude of the floral axis (2.66%). At last, in case of leaf length, the number of flowers and days to flowering, the contribution of lines, testers and L×T interaction has found almost same margin (Table 3).

Figure 1 Comparative mean performance of Parents (Lines and Testers) and L×T hybrids for studied traits
 PHT=Plant height, STD=Stem diameter, NOL=Number of leaves, LL=Leaf length, LW=Leaf width, NOF=Number of flower, DTF=Days to flowering, FLD=Diameter of the flower, BL=Bud length, AFA=Attitude of the floral axis

Table 2 ANOVA for L×T analysis

Sources of variation	d.f.	The mean sum squares of studied quantitative traits									
		PHT	STD	NOL	LL	LW	NOF	DTF	FLD	BL	AFA
Rep.	2	2.13ns	0.10ns	2.37ns	0.44ns	0.00ns	0.02ns	9.43ns	0.52ns	0.47*	2.27ns
Cross	14	164.73**	1.20**	92.84**	4.19**	0.43**	0.49**	68.37**	2.82**	1.33**	174.75**
Lines	4	389.72**	1.14**	239.00**	5.86**	0.51**	0.52**	77.84**	4.87**	2.53**	464.24**
Tester	2	158.59**	2.48**	40.56**	8.96**	1.44**	1.08**	169.92**	1.26*	3.64**	262.20**
L×T	8	53.78**	0.910**	32.82**	2.17**	0.14**	0.32**	38.24**	2.18**	0.16ns	8.15ns
Error	28	1.23	0.07	2.44	0.19	0.01	0.01	2.93	0.23	0.10	13.57

**and * significant at 1%and5% level of significance respectively.ns denotes non-significant.

Table 3 Proportional contribution of Lines, Testers and Line×Tester interaction

Parameters	PHT	STD	NOL	LL	LW	NOF	DTF	FLD	BL	AFA
Contribution of Lines	67.59	27.03	73.55	39.91	34.16	30.75	32.53	49.29	54.30	75.90
Contribution of Testers	13.75	29.62	6.24	30.50	47.50	31.86	35.50	6.38	39.00	21.43
Contribution of LXT	18.66	43.35	20.21	29.59	18.04	37.40	31.97	44.32	6.71	2.66

Table 4 Estimation of genetic component for 10 studied quantitative traits

Parameters	PHT	STD	NOL	LL	LW	NOF	DTF	FLD	BL	AFA
$\sigma^2A(VA=4\sigma^2GCA)F=1$	7.8453	0.0205	4.2434	0.1431	0.0208	0.0119	2.1300	0.0448	0.0833	11.7803
$\sigma^2D(VD=4\sigma^2SCA)F=1$	17.5168	0.2786	10.1305	0.6602	0.0406	0.0999	11.7720	0.6526	0.0195	-1.8065

Table 5.1 GCA effects of lines and testers for studied traits

Parents (Lines)	GCA with SE									
	PHT	STD	NOL	LL	LW	NOF	DTF	FLD	BL	AFA
L1(gi)	4.30**	0.56**	6.84**	-0.09ns	0.03ns	0.37**	0.24ns	-0.61**	-0.37**	1.51ns
L2	6.49**	-0.11ns	-4.18**	0.44**	0.31**	0.00ns	-2.87**	0.98**	0.61**	11.92**
L3	-7.98**	-0.25*	-2.93**	0.72**	0.07ns	-0.23**	-2.29**	-0.85**	-0.70**	-5.58**
L4	3.33**	0.12ns	4.22**	-1.34**	-0.35**	0.06ns	4.57**	0.24ns	0.33**	-3.91**
L5	-6.14**	-0.32**	-3.95**	0.27ns	0.08*	-0.20**	0.35**	0.23ns	0.13ns	-3.94**
SE	0.3704	0.0911	0.5204	0.1464	0.0396	0.0443	0.5709	0.1606	0.1044	1.2278

Table 5.2 GCA effects of lines and testers for studied traits

Parents (Testers)	GCA with SE									
	PHT	STD	NOL	LL	LW	NOF	DTF	FLD	BL	AFA
T1(gi)	0.55ns	-0.03ns	-1.89**	0.64**	0.07*	-0.21**	-3.88**	0.33*	0.28**	2.42*
T2	-3.49**	-0.39**	0.82ns	0.22ns	-0.34**	-0.09*	2.05**	-0.15ns	0.29**	-4.83**
T3	2.94**	0.42**	1.07*	-0.86**	0.27**	0.30**	1.84**	-0.18ns	-0.57**	2.41*
SE	0.2869	0.0706	0.4031	0.1134	0.0307	0.0343	0.4422	0.1244	0.0809	0.9510

**and * significant at 1% and 5% level of significance respectively. ns denotes non-significant.

3.4. General combining ability (GCA) effects

The estimated GCA effects of both female (lines) and male (testers) parents (Table 5.1 and 5.2 respectively) illustrated that none of the parents showed highly significant for all studied quantitative traits. Out of 5 lines, the performance of L2 i.e. (Stu × W) × AF1-6 for studied quantitative traits has found outstanding with highly significant GCA effects in positive direction for seven traits while rest of the traits i.e. stem diameter, the number of leaves and the number of flowers has found non-significant. Likewise, (L1) i.e. (AF × Ad) × HU sel.3 has shown highly significant GCA effects for plant height, leaf length, leaf width and the number of flowers while its GCA effects for the remaining traits found to be either non-significant or significant in negative direction. In another hand, line3 (L3) has demonstrated negatively significant GCA effects for eight traits.

Likewise, in the case of male parents (testers), T1 (Julius-19) has represented significant GCA effects for 6 traits besides non-significant GCA effects for plant height and stem diameter and significant GCA effects in negative direction for number of leaves and the number of flowers.

While T3 (12-1-2) L3 had possessed significant GCA effects for 6 traits, non-significant GCA effects for flower diameter and significant GCA effects in negative direction for leaf length, days to

flowering and bud length. At last T2 (WT-5) had shown all studied traits either non-significant or significant in negative direction.

3.5. The specific combining ability (SCA) effects and ranking of the L × T hybrids

The SCA is very important to determine whether particular crosses are superior or not. Moreover, SCA effects comprised both dominance and epistasis gene effects. The estimated SCA effects demonstrated that none of the L × T hybrids showed significant SCA effects for all studied quantitative traits (Table 6). Out of 15 L × T hybrids so far employed for studied quantitative traits in this experiment, on the basis of significant SCA effects in positive direction around half of them are found good performer. The L × T hybrids, L4 × T3 has possessed significant SCA effects in positive direction for plant height, stem diameter, number of leaves, leaf length and days to flowering while rest 5 of the traits are found non-significant. Another L × T hybrid that had possessed significant SCA effects for 5 traits is L5 × T1. It had represented significant SCA effects for plant height, stem diameter, leaf width, days to flowering and flower diameter while its SCA effects for rest of the different five studied traits has demonstrated non-significant. Likewise, other groups of L × T hybrids, L2 × T1, L2 × T2 and L3 × T2 had possessed significant SCA effects for 4 studied quantitative traits and had possessed non-significant SCA effects for rest of the studied traits and The L × T hybrids (L3 × T3) had

Table 6 SCA effects of L×T hybrids for studied traits

L×T hybrids(Cross)	PHT	STD	NOL	LL	LW	NOF	DTF	FLD	BL	AFA
L1×T1	-4.70**	-0.26ns	-1.61ns	-0.73**	-0.16*	-0.19*	-0.76**	-0.07ns	-0.01ns	0.49ns
L1×T2	1.24ns	-0.03ns	0.01ns	0.76**	0.18*	-0.21*	1.97ns	-0.05ns	0.01ns	-1.01ns
L1×T3	3.47**	0.29ns	1.59ns	-0.03ns	-0.02ns	0.40**	1.79ns	0.12ns	0.00ns	0.51ns
L2×T1	1.37**	0.36*	3.08**	-0.03ns	-0.11ns	0.28**	4.02**	0.15ns	-0.02ns	1.33ns
L2×T2	5.21**	0.23ns	2.80**	-0.00ns	0.14ns	0.16*	-2.75**	-0.43ns	-0.24ns	-2.67ns
L2×T3	-6.59**	-0.60**	-5.88**	0.04ns	-0.03ns	-0.44**	-1.27ns	0.28ns	0.26ns	1.34ns
L3×T1	0.68ns	-0.45**	-0.61ns	0.26ns	-0.03ns	-0.09ns	0.61ns	-0.82**	-0.14ns	-1.17ns
L3×T2	-1.21ns	-0.22ns	-0.49ns	0.75**	-0.18*	-0.11ns	-2.16*	0.76*	0.41*	2.33ns
L3×T3	0.52ns	0.67**	1.09ns	-1.01**	0.21**	0.20*	1.55ns	0.06ns	-0.26ns	-1.16ns
L4×T1	-0.46ns	-0.17ns	-0.55ns	0.19ns	0.08ns	0.12ns	2.91**	-0.70*	0.09ns	-0.34ns
L4×T2	-2.59**	-0.18ns	-3.36**	-1.28**	-0.20**	-0.20*	-1.59ns	0.08ns	-0.12ns	0.66ns
L4×T3	3.05**	0.35*	3.92**	1.09**	0.12ns	0.08ns	-1.31ns	0.62*	0.04ns	-0.32ns
L5×T1	3.11**	0.52**	-0.32ns	0.31ns	0.22**	-0.12ns	-3.77**	1.44**	0.09ns	-0.31ns
L5×T2	-2.65**	0.20ns	1.04ns	-0.23ns	0.06ns	0.36**	4.53**	-0.35ns	-0.06ns	0.69ns
L5×T3	-0.45ns	-0.72**	-0.72ns	-0.09ns	-0.28**	-0.24**	-0.76ns	-1.09**	-0.03ns	-0.38ns
SE	0.6416	0.1578	0.9013	0.2535	0.0687	0.0767	0.9888	0.2781	0.1808	2.1266

**and * significant at 1%and5% level of significance respectively.ns denotes non-significant.

shown significant SCA effect for stem diameter, leaf width and number of flower.

According to the method of Arunachalam & Bandyopadhyay (1979), the ranking of the different L×T hybrids has been prepared on the basis of traits and presented in Table 7.

4 Discussion

In this study, the mean performance of Lines (three way-cross F₁s), Testers (donor CVs/breeding lines) and their L×T hybrids demonstrated significant differences for the studied quantitative traits (Rai et al., 2018; Rai et al., 2019). Montazeri et al. (2014) in Rice and Kumari et al. (2015) in Pea had also been found significant variation among the studied traits of lines, testers and L×T hybrids. Furthermore, the main source of variation among the studied genotypes is their genetic background. Since the time of flowering is a major concern for the Lily grower in Korea (Kang et al., 2013) to earn the hard cash due to off-season supply of cut flower in the market and the breeding trend of making interspecific hybridization (Younis et al., 2014), the single cross F₁s and thereby three-way cross F₁s had been prepared including at least one genotype from *L. longiflorum* and two genotypes had included from *Lilium*×*formolongi*. Besides, donors (testers) has also chosen purposefully keeping in mind their quantitative traits (Table 1), the

genetic makeup of three way-cross F₁s; one clonal line of *Lilium*×*formolongi* (i.e.Julius-19), one clonal line of *L. longiflorum* CV White Tower (i.e.WT-5) and one clonal line of *L. longiflorum* breeding line 12-2-2 to introduce the earliness to the L×T hybrids so that they can use for forcing inside the greenhouse for off-season supply to the flower market. On the other hand, the ANOVA for L×T analysis showed significantly different for all studied quantitative traits among the genotypes under investigation that means prevailing genotypic variation among them indicated the rationality of conducting this experiment. Genetic variability is the key instrument for any breeder to execute any breeding and improvement scheme for particular quantitative traits. Though morphological observation is one of the basic criteria for the making decision about selection and improvement of particular quantitative traits but also have to consider other genetic analysis.

The study of L×T combining ability analysis represents another important insight is related to GCA and SCA effects which comprises the relative measure of additive and non-additive gene action which involved in the inheritance of particular quantitative traits. Out of the 10 studied quantitative traits, only bud length and the attitude of the floral axis demonstrated the prevailing of the additive type of gene action. It opens the possibility of further backcross breeding for these traits choosing L×T hybrids; (L4×T3), (L5×T1), (L2×T1), (L2×T2) and (L3×T2) mother line

Table 7 Ranking of superior specific combiner for particular quantitative traits.

Traits	L×T hybrids	SCA effect	GCA effect		Mean Performance	Combination
			Female	Male		
1.PHT (cm)	L1×T3	3.47**	4.30**	2.94**	75.00	H×H
	L2×T1	1.37**	6.49**	0.55 ns	72.70	H×H
	L2×T2	5.21**	6.49**	-3.49**	72.50	H×L
	L4×T3	3.05**	3.33**	2.94**	73.60	L×H
	L5×T1	3.11**	-6.14**	0.55 ns	61.80	L×H
2. STD (mm.)	L2×T1	0.36*	-0.11ns	-0.03ns	6.71	H×H
	L3×T3	0.67**	-0.25*	0.42**	7.32	L×H
	L4×T3	0.35*	0.12ns	0.42**	7.37	L×H
	L5×T1	0.52**	-0.32**	-0.03ns	6.66	L×H
3.NOL (no.)	L2×T1	3.08**	-4.18**	-1.89**	28.77	H×H
	L2×T2	2.80**	-4.18**	0.82ns	31.20	H×L
	L4×T3	3.92**	4.22**	1.07*	40.97	L×H
4.LL (cm.)	L1×T2	0.76**	-0.09ns	0.22ns	10.10	H×L
	L3×T2	0.75**	0.72**	0.22ns	10.90	L×L
	L4×T3	1.09**	-1.34**	-0.86**	8.10	L×H
5.LW (cm.)	L1×T2	0.18*	0.03ns	-0.34**	2.27	H×L
	L3×T3	0.21**	-0.07ns	0.27**	2.80	L×H
	L5×T1	0.22**	0.08*	0.07*	2.77	L×H
6.NOF (no.)	L1×T3	0.40**	0.37**	0.30**	3.30	H×H
	L2×T1	0.28**	0.00ns	-0.21**	2.30	H×H
	L2×T2	0.16*	0.00ns	-0.09*	2.30	H×L
	L3×T3	0.20*	-0.23**	0.30**	2.50	L×H
7.DTF (days)	L2×T2	-2.75**	-2.87**	2.05**	105.27	H×L
	L3×T2	-2.16*	-2.29**	2.05**	106.43	L×L
	L5×T1	-3.77**	0.35ns	-3.88**	101.53	L×H
8.FLD	L3×T2	0.76*	-0.85**	-0.15ns	49.72	L×L
	L4×T3	0.62*	0.24ns	-0.18ns	50.64	L×H
	L5×T1	1.44**	0.23ns	0.33*	51.96	L×H
9.BL	L3×T2	0.41*	-0.70**	0.29**	15.80	L×L

**and * significant at 1%and5% level of significance respectively.ns denotes non-significant.

D.F. =Degree of freedom, GCA=General combining ability, SCA=Specific combining ability, SE=Standard error, L×T=Line×Tester, H×H=High×High, H×L=High×Low, L×H=Low×High, L×L=Low×Low, PHT=Plant height, STD=Stem diameter, NOL=Number of leaves, LL=Leaf length, LW=Leaf width, NOF=Number of flower, DTF=Days to flowering, FLD=Diameter of the flower, BL=Bud length, AFA=Attitude of the floral axis

and T1 (i.e.Julius-19) as the donor (since GCA effects of T1 shows significant in the positive direction for both of these traits). In contrast with the findings of Song et al., 2004; Rai et al., 2018 and Rai et al.,2019 plant height, stem diameter, the number of leaves,

leaf length, the number of flowers, days to flowering and flower diameter represented prevalence of non-additive gene action thereby indicated the importance of heterosis breeding followed by selection for these quantitative traits. The contrast gene action

estimation for particular quantitative traits is normal primarily that depends upon the pedigree of the selected lines and testers. Furthermore, Song et al. (2004) have employed Single cross F₁s in their diallel analysis while Rai et al. (2018) and Rai et al. (2019) have used Single cross F₁s and Double-cross F₁s as mother lines in their lines×tester analysis.

The most important insight for the selection of particular cross combinations (hybrids) depending upon the choice of particular traits is their SCA effects. The SCA effects represent both the dominance and epistatic effects of a hybrid. In this experiment out of 15 L×T hybrids none of the hybrids demonstrated significant value in a positive direction for all studied quantitative traits. Though SCA effects reflect that L×T hybrids (L4×T3), (L5×T1), (L2×T1), (L2×T2), (L3×T2) and (L3×T3) can be used for the further selection process. The previous results of Narasimhamurthy & Gowda, (2013) in Tomato, Kumari et al. (2015) in Pea, Rai et al. (2018) and Rai et al. (2019) in *Lilium × formolongi* has been so far reported that the high specific combiner is the outcomes of the all three types of combination of general combiner parents viz. high×high, low×low and high ×low that means not always comes only from the high×high general combiner parents. There are another evidence that according to the Sharma et al. (2013), in the majority of the cases, those crosses exhibiting high SCA effects in a positive direction, were found to have both or one of the parent as good general combiner for the studied traits exhibiting non-additive gene action in the genetic control. Those parents having high GCA effects in positive direction but sometimes produced low SCA effect is the clear evidence of the lack of complementation of the parental genes. Likewise those parents having low GCA effects produced hybrids with high SCA effects is the clear evidence of complementary gene action for some traits (Kumari et al., 2015).

Conclusions

The significant variability prevailing in the lines (three way-cross F₁s), testers (donor CVs/breeding line) and their L×T hybrids for studied quantitative traits has provided the significance of this experiment. The three way-cross F₁s; (Stu×W) × AF₁-6 (L2) and (AF×Ad) × HU-3(L1) can be used as mother lines for seed production inside the plastic house. For this; Julius-19(T1) and 12-1-2(T3) can be used as effective donors. Furthermore, L×T hybrids; (L4×T3), (L5×T1), (L2×T1), (L2×T2) and (L3×T2) can be used as promising hybrids and some lines of this genotypes can select and either register as a new cultivar or can be employed for further breeding program.

Acknowledgements

This work was carried out with a grant of a Golden Seed Project, the Ministry of Agriculture, Food and Rural Affairs, Republic of

Korea (Project No.213007-05-4-SBN10), a grant from the Germplasm Reservation Center Program, Rural Development Administration, Republic of Korea (Project PJ015202012020)

Conflict of interest

All the authors declare that there is no conflict of interest about the contents of the article.

References

- Allard RW, Bradshaw AD (1964) Implications of genotype-environmental interactions in applied plant breeding. *Crop Science* 4:503-508.
- Arunachalam V, Bandyopadhyaya A (1979) Are "multiple cross multiple pollen hybrids" an answer for productive population in *Brassica campestris* var. brown sarson ?1. Methods for studying 'mueromphs'. *Theoretical and Applied Genetics* 54:203-207.
- Bakhshaei M, Khosravi S, Azad P, Bagheri H, Van Tuyl JM (2016) Biotechnological advances in Lilium. *Plant Cell Reports* 35:1799-1826.
- Goo DH, Lim JH, Rhee HK, Cho HR, Kim YJ, Chung HY, Kim KW (2003) Lily Culture.No.133 Rural Development Administration, Suwon, Korea (in Korean).
- Grassotti A, Gimelli F (2011) Bulb and cut flower production in the genus Lilium: current status and the future. *Acta Horticulture* 900.
- Ho CW, Jian WT, Lai HC (2006) Plant regeneration via somatic embryogenesis from suspension cell cultures of Lilium × formolongi Hort.using a bioreactor system. *In vitro Cellular and Developmental Biology-Plant* 42:240-246.
- Kang YI, Joung HY, Goo DH, Choi YJ, Choi MP, An HR, Ko JY, Choi KJ, Lee KH, Hong KW (2013) A Survey on Cut Flower Cultivar Trends and Horticultural Status of Lilies (Lilium Hybrids) in South Korea. *Hort Technology* 23:629-634.
- Kemphorne O (1957) An Introduction to Genetic Statistics. John Wiley and sons Inc. New York, U.S.A.
- Kumari J, Dikshit HK, Singh B, Singh D (2015) Combining ability and character association of agronomic and biochemical traits in pea (*Pisum sativum* L.). *Scientia Horticulturae* 181:26-33.
- Lucidos JGB, Kwang R, Younis A, Kim CK, Hwang YJ, Son BG, Lim KB (2013) Different day and night temperatures responses in Lilium hansonii in relation to growth and flower development. *Horticulture, Environment, and Biotechnology* 54:405-41.
- Manivannan N (2014) TNAU-STAT-Statistical Package. Retrieved from <https://sites.google/site/tnaustat> access on 19December, 2019.

- Matthews V (2007) The International Lily Register and Checklist. RHS London, Fourth Edition.
- McRae E (1998) Lily species. In: Lilies Timber Press, Portland, Ore. USA, pp.319-330.
- Montazeri Z, Jelodar NB, Bagheri N (2014) Genetic Dissection of Some Important Agronomic Traits in Rice Using Line $\times$ Tester Method. International Journal of Advanced Biological and Biomedical Research 2:181-191.
- Narasimhamurthy YK, Gowda PHR (2013) Line x Tester analysis in Tomato (*Solanum lycopersicum* L.): Identification of superior parents for fruit quality and yield-attributing traits. International Journal of Plant Breeding 7:50-54.
- Nduwumuremyi A, Tongoona P, Habimana S (2013) Mating designs: helpful tool for quantitative plant breeding analysis. Journal of Plant Breeding and Genetics 1:117-129.
- Rai R, Shrestha J, Kim JH (2018). Combining Ability and Gene Action Analysis of Quantitative Traits in *Lilium* $\times$ *formolongi*. Journal of Agricultural, Life and Environmental Sciences 30:131-143.
- Rai R, Shrestha J, Kim JH (2019) Line x Tester analysis in *Lilium* $\times$ *formolongi*: Identification of superior parents for growth and flowering traits. SAARC Journal of Agriculture 17:175-187.
- Rashid M, Cheema AA, Ashraf M (2007) Line x tester analysis in basmati rice. Pakistan Journal of Botany 39: 2035-2042.
- Roh MS (2002) New Production Technologies of *Lilium* - Prospects and Limitations. Korean Journal of Horticultural Science and Technology 20:174-190.
- Schnell FW (1975) Type of variety and average performance on hybrid maize. Zeitschrift fuer Pflanzenzuechtung 74:177-188.
- Sharma BB, Sharma VK, Dhakar MK, Punetha S (2013) Combining ability and gene action studies for horticultural traits in garden pea: A review. African Journal of Agricultural Research 8:4718-4725.
- Sharma JR (2006) Statistical and Biometrical techniques in Plant breeding (First ed.) New Age International, New Delhi, India.
- Song CY, Park SC, Lee JS, Kim YA, Kim JH (2004) Correlation and combining ability of plant growth and flowering in F1 hybrids by diallel cross in *Lilium formolongi* and *L. formosanum*. Korean Journal of Horticultural Science and Technology 22:107-113.
- Van Tuyl JM, Arens P (2011) *Lilium*: Breeding History of the modern cultivar assortment. Acta Horticulturae 900:223-230.
- Weatherspoon JH (1970) Comparative Yields of Single, Three-Way, and Double Crosses of Maize. Crop science 10:157-159.
- Xuan Y, Davaasuren YE, Kyung HY, Kim JH (2005) Recovery of quantitative cut flower characters in backcross generation of *Lilium formolongi*. Korean Journal of Flower Research Society 13:268-272.
- Younis A, Hwang YJ, Lim KB (2014) Classical vs. modern genetic and breeding approaches for lily (*Lilium*) crop improvement: a review. Flower Research Journal 22:39-47.