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LINE×TESTER ANALYSIS IN *LILIUM*×*FORMOLONGI:* IDENTIFICATION OF SUPERIOR PARENTS FOR GROWTH AND FLOWERING TRAITS

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ABSTRACT

An experiment was carried out using eight double cross F_1s (DCF_1s) as lines and two testers to obtain sixteen Line ×Tester hybrids for the evaluation of major growth and flowering traits thereby to understand the breeding potentiality of the parental lines. The performance of growth and flowering traits of all twenty six genotypes were evaluated in a randomized complete block design (RCBD) with three replications. The analysis of variance (ANOVA) showed all the genotypes were significantly different for all agro-morphological traits under study. The estimated general combining ability (GCA) effects showed that parent 5(P5), were superior for plant height, stem diameter, number of leaves, leaf length, days to flowering and attitude of floral axis followed by parent 2(P2) for leaf length, leaf width, days to flowering and attitude of floral axis and parent7 (P7) for number of flower, flower diameter, bud length and attitude for floral axis. Likewise; tester 1 found to be best combiner with significant GCA effect for almost all growth and flowering traits (except number of leaves). The plant height, leaf width, number of flower and attitude of floral axis showed prevailing additive gene action while stem diameter, number of leaves, leaf length, days to flowering, flower diameter and bud length indicated predominance of dominance gene action. We can conclude that parent P5, parent P2 and parent P7 can be used as good mother lines and the tester 1, as good donor for the seed production.

Keywords: General Combining Ability (GCA), Line×Tester Analysis, Line ×Tester Hybrids, *Lilium*×*formolongi,* Specific Combining Ability (SCA)

INTRODUCTION

Lily (Lilium L., 2n = 2x = 24), comprising members of the Liliaceae family, is one of the most popular groups of ornamental bulbous monocot outcrossing perennial herbs worldwide due to their incomparable beauty and commercial importance (Shahin et

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al., 2012). Many commercial cultivars have been produced by interspecific hybridization (van Tuyl and Arens, 2011). The *Lilium* × *formolongi* hort; an interspecific hybrid of *L. formosanum* and *L. longiflorum*, is a popular commercial cut flower in Korea, Japan and China (Ho et al., 2006). The production of hybrid lilies was done by hybridization of two single cross hybrids. Double cross (DC) hybrids can be produced by crossing two unrelated single cross (SC) hybrids. Firstly, two pairs of inbred lines crossed to produce SC hybrids and secondly, those SC hybrids need to cross to produce DC hybrids are the usual two steps needed for the development of DC hybrids. The DC hybrids with comparison to the SC hybrids, have wider genetic diversity thereby possess ecologically wider spans and are more adaptable to environmental conditions as a mixture of genotypes have better chances of success to cope up with varied environmental conditions (Ekinci et al., 2016). The double cross was revealing high potentiality it could be an indication of differences in the dominant favorable alleles distributed among the two single cross parents is different (El-hashash, 2013). DC hybrid F_1s are stable and intermediate in terms of performance for different growth and flowering traits. The genotypic performance of DC hybrid F_1s of *L.*×*formolongi* demonstrated early flowering and middle plant height and moderate performance for some important growth and flowering traits. The attitude of the floral axis as considered one of the most important cut flower traits. As most growth and flowering traits of the DCF_1s are more stable than $SCF_{1;}$ some important cut flower traits need to improve to fulfill consumer's need. In this context, some clonal lines of donor cultivar and breeding lines using as tester(whose pedigree are not related to lines) an attempt has been made to produce the special cross F_1s using double cross F_1s as mother lines with the application of lines × Tester mating design.

Line × Tester mating design was first proposed by Kempthorne (Sharma, 2006). Among the different breeding tools, the line × tester analysis is used in both self as well as cross-pollinated crops to estimate general and specific combing abilities of specific traits and to determine favorable parents and cross. This design emphasizes hybridization between lines (f) and wide based testers in one to one fashion of mating generating f × m = fm hybrids (Sharma, 2006). In this way, on the basis of GCA we can select favorable parents (both male and female), as well as on the basis of the estimated SCA, we can determine the appropriate hybrids (cross combination).The objectives of the present study were therefore to examine the combining abilities patterns of selected Lilium × formolongi in a line × tester analysis, to assess genetic parameters of some agronomic traits, to determine superior candidates for promising hybrid cross combinations.

MATERIALS AND METHODS

Preparation of plant material, generation of crosses and field experiment:

The plant material preparation, generation of crosses and field experiment has been

carried out in KNU, experimental farm in Chuncheon, Kangwon-do, South Korea during 2015-17. The experiment area is located at 37°52´N latitude and 127°44´E longitudes. The area located in a basin formed by the Soyang River and Han River. The area lies at 99 m from msl and annual precipitation appears 1347.3 mm. The experiment area demonstrates high temperature and humidity during the summer and coldness and dryness during the winter (Kwon et al., 2016). The F_1s (single cross F_1s) seeds were obtained from KNU, department of Horticulture, Floricultural breeding laboratory in 2015, double cross hybridization, selection of double cross F_1s lines and L×T mating has been carried out in the succeeding year continuously. In this way, in 2017 we have prepared the seeds of 26 genotypes including the 8 lines, 2 testers and 16 L×T hybrids for the execution of the experiment. The details of parental materials used for this experiment are given in Table 1.

The seedling has been prepared inside the plastic house during January to April (Goo, 2008). Since the temperature of chuncheon in winter becomes very cold (Kwon et al., 2016), inside the plastic house night and day temperature has been maintained 15±3°C and 25±3°C as described by Rai et al. (2018). In the third weeks of April prepared seedling has been transplanted in main field laying out in RCBD (Randomized complete block design) with 3 replication to evaluate the growth and flowering traits. The seedlings of all genotypes has been grown randomly in multiple bed plots as block consisting 3 cm long and 1m in width. The seedlings has been transplanted maintaining row to row and plant to plant equal distance of 12.5 cm using the mulching plastic (black color) available in the market. In each replication for all treatments 1 m^2 area has been provided to maintain the seedling population 64. The distance between the beds has been maintained 80 cm to ease for intercultural operation. Since chuncheon represents very hot and humid weather during summer so well provision of irrigation has been provided fixing the 4 drip irrigation hose as length wise of bed at the equal distance of 25 cm before covering the bed with mulching plastic. Before making beds, the land has been well prepared ploughing the 2-3 times and recommended N, P, K containing fertilizer has been provided as per the recommended doses. The weeding and application of insecticide and fungicide has been provided with the interval of 2-3 weeks from the transplanting of seedling (3^{rd} week of April) until harvesting of bulbs (usually first week of November).

Morphological observation of studied growth and flowering traits has been taken during main season of flowering i.e. July-August; sampling12 plants from each replication. At the last, crossing procedure for preparing plant material, seedling preparation and morphological observation of studied traits has been carried out following Rai et al. (2018).

Statistical analysis

The ANOVA for L×T analysis was carried out on the basis of method as suggested by Kempthorne (1957). The estimation of components of genetic variances, the estimation of combining ability effects, specific combining ability effects, the

standard errors for testing the significance of GCA and SCA effects and the proportional contribution of lines, testers and line × tester interactions to the total variance. All these calculations were performed with the help of the software package TNAUSTAT statistical packages (Manivannan, 2014). On the basis of overall GCA status of their parents involved, the ranking of the best specific combiner has been arranged for the particular growth and flowering traits adopting the method as outlined by Arunachalam and Bandyopadhyay (1979).

RESULTS

The mean performance of parents and L×T hybrids

he mean performance of lines, testers and L×T hybrids showed genetic variability among those genotypes for studied growth and flowering traits (Fig. 1). Among the lines, testers and L×T hybrids; testers demonstrated the outstanding performance for all most all traits besides intermediate performance in flower diameter. Likewise lines possessed outstanding result for flower diameter and stem diameter, number of leaves, leaves width and number of flower with lowest performance for days to flowering and attitude of floral axis. L×T hybrids showed intermediate performance i.e. in between the testers and lines for almost traits.

ANOVA for L×T analysis

The ANOVA for L×T analysis (table 2) demonstrated that there were significant differences among the L×T hybrids (crosses) for all studied growth and flowering traits. Likewise, there were significantly different among the both lines and testers for all studied traits. The interaction effect between line and tester was significant for almost studied traits (except attitude of floral axis).

Gene action and contribution of line, tester and line x tester interaction

The plant height, leaf width, number of flower and attitude of floral axis demonstrated additive gene action while remaining traits viz.stem diameter, number of leaves, leaf length, days to flowering, flower diameter and bud length demonstrated dominance gene action (Table 4). As shown in table 3, contribution of lines were recorded highest for number of leaves (81.69%) while lowest contribution of lines were 13.78% for days to flowering. Likewise, contribution of testers was found highest 76.20% for days to flowering and lowest 3.73% for number of leaves. Lastly, contribution of L×T interaction was found highest 27.95% for leaf length and lowest 1.07% for attitude of floral axis.

Table 1. List of lines (DCF_1s) and testers (CV/breeding line), pedigree and traits remarks

S.N	Genotypes	Pedigree	Remarks
(A)Lines			
1.	(Stu× W)-9 × (AugE×BT)-6 (P1)	DCF_1 of L.Fl.SCF_1s(Stu × W)-9 &(AugE×BT)-6	M-T,upward facing ,middle fl. time
2.	(Stu × W)-9 ×57-6 (Aug×AugE) (P2)	$DCF_1$of L.Fl.SCF_1s(Stu ×W)-9&(57-6 (Aug×AugE)	Taller ,upward ,strong but late
3	(Stu × W)-9 × (AugE×IS)-1(P3)	DCF_1 of L.Fl.SCF_1s(Stu × W)-9 &(AugE×IS)-1	Middle side -up dir, very early
4	(Stu × W)-9 × (AugE×Gelria)-16(P4)	DCF_1 of L.Fl.SCF_1s(Stu ×W)-9 &(AugE×Gelria)-16	M-T,up dir.& strong
5	(Stu × W)-9 × 58.15(AugE×J) (P5)	DCF_1 of L.Fl.SCF_1s(Stu × W)-9 &58.15(AugE×J)	Middle, early ,strong and up dir.
6	(Stu × W)-9 × (WT×AugE)-9(P6)	DCF_1 of L.Fl.SCF_1s(Stu × W)-9 &(WT×AugE)-9	Middle PHT,early &upward
7	(Stu × WT)-4 × (J×G)-1(P7)	DCF_1 of L.Fl.SCF_1s(Stu × W)-9 &(J×G)-1	Mid PHT& fl. Time and Upward
8	(AF× 12-1)-8 × 57-6 (Aug×AugE) (P8)	DCF_1ofL.Fl.SCF_1s(AF ×12-1)-8 &57-6 (Aug×AugE)	Taller,upward,M-L fl. time
(B) Testers			
1.	R.H.-4(P9)	Selected clone of L.fl. CV Raizan Herald(RH)	Middle PHT,v early & up-near up
2	HU-2(P10)	Selected clone of L.L breeding line Hinomoto Up	M-T,early& near up

Abbr. P1=Parent 1,……., P10=Parent 10, SCF1s=Single cross F_1s, DCF1s=Double cross F_1s,L.fl=Lilium ×formolongi,LL=Lilium longiflorum,CV=Cultivar,M-T=Middle-Tall,PHT=Plant height

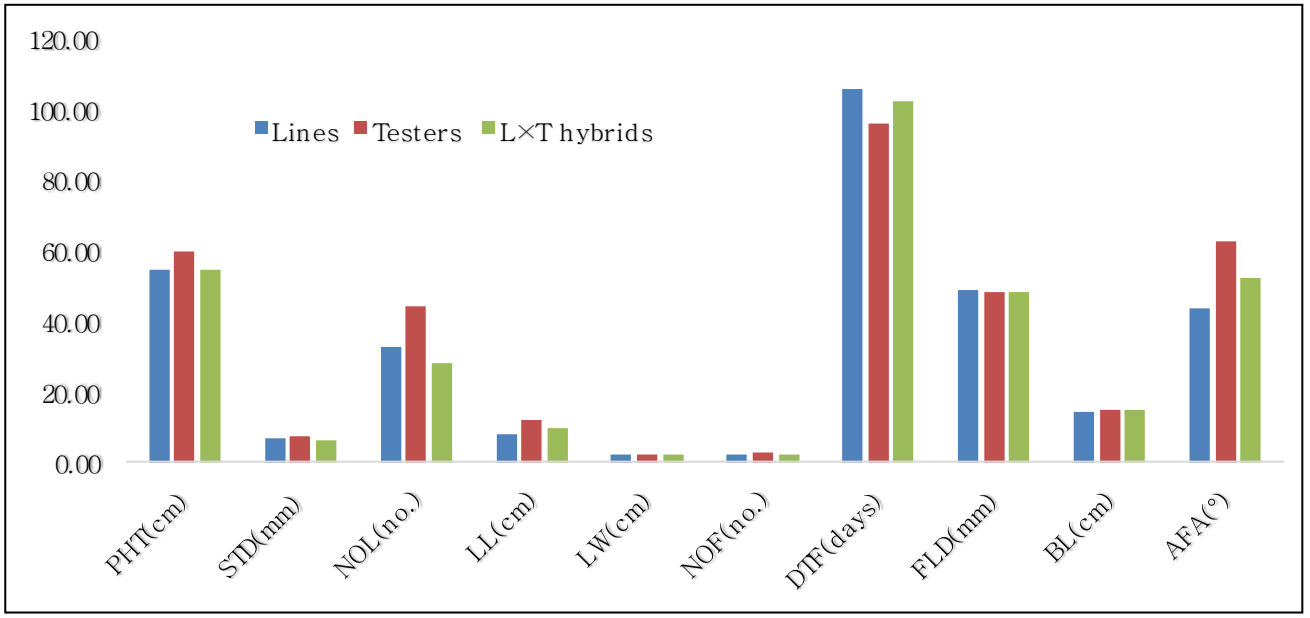

Figure 1. Comparative mean performances of Line, Testers and L×T hybrids for growth and flowering traits

Table 2. ANOVA for L×T analysis

Sources of variation	d.f.	Mean Sum Square of ……..									
		PHT	STD	NOL	LL	LW	NOF	DTF	FLD	BL	AFA
Replications	2	0.2425ns	0.0099ns	2.1102ns	0.0419ns	0.0008ns	0.0019ns	1.1502ns	0.1143ns	0.0058ns	45.70ns
Cross	15	99.91**	0.3552**	37.62**	2.70*	0.1049**	0.0979**	124.16**	2.0558**	3.80**	320.13**
Lines(c)	7	71.84**	0.4781**	65.85**	3.18**	0.0631*	0.1347*	36.67**	1.1415**	5.57**	430.53**
Tester(c)	1	978.31**	1.39**	21.06**	6.90**	1.050**	0.4219**	1419.18**	15.6751**	8.16**	1737.01**
L×T (C)	7	2.4893**	0.834**	11.74**	1.61**	0.0116**	0.0147**	26.66**	0.7645**	1.41**	7.32ns
Error	30	0.2878	0.0230	1.2073	0.1157	0.0008	0.0019	0.8347	0.1389	0.1532	19.14

ANOVA=Analysis of Variance, d.f. =degree of freedom ,PHT-Plant height,STD-Stem diameter,NOL-Number of leaves,LL-Leaf length,LW-Leaf width,NOF-Number of flowers,DTF-days to flowering,FLD-Diameter of flower,BL-length of bud and AFA-attitude of floral axis, L×T (C)=Line×Tester(Cross)

**and *Significant at 1%and 5% level of significance respectively

Table 3. Proportional contribution of Lines, Testers and their interaction for studied growth and flowering traits

Parameters	PHT	STD	NOL	LL	LW	NOF	DTF	FLD	BL	AFA
Contribution of Lines (L)	33.56	62.81	81.69	55.02	28.06	64.24	13.78	31.81	68.32	62.76
Contribution of Testers (T)	65.28	26.23	3.73	17.03	66.76	28.74	76.20	50.83	14.30	36.17
Contribution of LXT	1.16	10.96	14.57	27.95	5.18	7.02	10.02	17.35	17.38	1.07

General combining ability effects

The estimation of GCA effects of lines (table 5.1) and testers (table 5.2) indicated that, line 5, (Stu× W)-9 × 58.15 (AugE×J) has significant GCA effect for 6 traits viz. Plant height, stem diameter, number of leaves, leaf length, days to flowering and attitude of floral axis. While line 2, (Stu× W)-9 × 57.6 (Aug×AugE) has significant GCA effect for 5 traits viz. leaf length, leaf width, days to flowering, bud length and attitude for floral axis and line7, (Stu× WT)-4 × (J×G)-1 has demonstrated significant GCA effect for 4 traits viz. Number of flower, flower diameter, bud length and attitude for floral axis etc. In case of testers, tester 1, (Stu× WT)-4 × (J×G)-1 (RH-4) found to be best combiner with significant GCA effect for almost all quantitative traits (except no of leaves)

Specific combining ability effects

The specific combining ability effects included both dominance and epistemic gene effects. It is very important indicator for the selection of particular cross combination i.e. cross hybrids. In this experiment we have observed that none of the cross combination have found superior performance for all the traits under study. It is the evidence that high specific combination were also obtained from High×Low and Low×Low general combiners not only directly resulted from the combination of High×High general combiners (Table 6). The cross combination having significant SCA effects in positive direction has been listed in the table 8 and ranked on the basis of the significant GCA effects of their parents on the basis of overall performance (Arunachalam and Bandyopadhyay, 1979).

DISCUSSION

L.× *formolongi* is an interspecific hybrid of *L. formosanum* and *L .longiflorum*. In *L.* × *formolongi* F_1 hybrids .The homogenous performance of the growth and flowering traits is essential to get the good price from cut flower market. For the selection of genotypically diverse parental line and to understand the gene action among the quantitative traits an experiment has been conducted including 8 double cross F_1s(DCF_1s) as lines and 2 testers as donor to obtain 16 special (L×T) hybrids . The mean performance of special Line×Tester hybrids demonstrated superior performance for important growth and flowering traits viz. days to flowering, bud length and attitude of floral axis. It is the proof of improvement of these growth and flowering traits with the application of testers. While other remaining traits remain more or less near to the performance of double cross F_1s (DCF_1s). Moreover mean performance of testers were higher for the traits of plant height, stem diameter, number of leaves, leaf length, leaf width, number of flowers, days to flowering and attitude of the floral axis, thereby proving the rationality of selecting those testers for the improvement of those traits to increase the value of cut flower in *L.* ×*formolongi* .All the genotypes used; demonstrated highly significant mean sum square value for all studied growth and flowering traits. Furthermore, Line×Tester hybrids also demonstrated significant mean sum square for almost studied traits indicated the significance of Line×Testers model for combining ability and gene action.

Table 4.Estimation of genetic component for studied growth and flowering traits

Parameters	PHT	STD	NOL	LL	LW	NOF	DTF	FLD	BL	AFA
Var of GCA	2.7677	0.0077	0.7350	0.0308	0.0026	0.0024	2.7701	0.0367	0.0679	8.8867
Var of SCA	0.7338	0.0201	3.5140	0.5008	0.0036	0.0043	8.6086	0.2085	0.4216	-3.9388
GCA:SCA ratio	3.7717	0.3830	0.2091	0.0615	0.7222	0.5581	0.3217	0.1760	0.1610	2.2581
σ^2A(VA=4σ^2GCA)F=1	5.5353	0.0154	1.4700	0.0616	0.0053	0.0047	5.5403	0.0734	0.1358	17.7734
σ^2D(VD=4σ^2SCA)F=1	0.7338	0.0201	3.5140	0.5008	0.0036	0.0043	8.6086	0.2085	0.4216	-3.9388

GCA=General combining ability,SCA=Specific combining ability,A=Additive,D=Dominance,L×T (C)=Line×Tester(Cross),F=breeding coefficient of crop

**and *Significant at 1%and 5% level of significance respectively

Table. 5.1. GCA effect of lines for 10 quantitative traits in special breeding

Parents	PHT	STD	NOL	LL	LW	NOF	DTF	FLD	BL	AFA
Lines1	-2.57**	-0.42**	-0.21ns	-0.32*	-0.14**	-0.24**	1.53**	-0.45**	-1.32**	-5.86**
Lines2	-0.41ns	-0.04ns	-3.75**	0.70**	0.21**	0.01ns	-1.80**	0.10ns	0.68**	9.77**
Lines3	-2.44**	-0.36**	-0.50ns	-1.20**	-0.04**	-0.14**	-3.12**	-0.68**	-1.55**	-11.48**
Lines4	-0.14ns	0.21**	-3.50**	0.11ns	-0.09**	-0.01ns	-1.22**	-0.47**	0.10ns	-8.98**
Lines5	8.26**	0.32**	3.99**	1.20**	-0.00ns	-0.11**	-1.24**	0.27ns	0.07ns	7.89**
Lines6	-0.42ns	0.25**	0.27ns	-0.09ns	0.01ns	-0.01ns	0.43ns	0.38*	0.82**	-3.98*
Lines7	-1.31**	-0.13*	-1.86**	0.08ns	0.01ns	0.26**	4.90**	0.71**	1.10**	5.39**
Lines8	-0.97**	0.16*	5.55**	-0.47**	0.01ns	0.01ns	0.53ns	0.13ns	0.10ns	7.27**
SE	0.2190	0.0619	0.4486	0.1388	0.0118	0.0177	0.3730	0.1521	0.1598	1.7861

Table 5.2.GCA effect of testers for 10 quantitative traits in special breeding

Parents	PHT	STD	NOL	LL	LW	NOF	DTF	FLD	BL	AFA
Testers 1	4.51**	0.17**	-0.66**	0.38**	0.15**	0.09**	-5.44**	0.57**	0.41**	6.02**
Tester2	-4.51**	-0.17**	0.66**	-0.38**	-0.15**	-0.09**	5.44**	-0.57**	-0.41**	-6.02**
SE	0.1095	0.0310	0.2243	0.0694	0.0059	0.0088	0.1865	0.0761	0.0799	0.8930

Line 1=(Stu× W)-9 × (AugE×BT)-6**, Line 2=**(Stu× W)-9 × 57.6(Aug×AugE)**, Line3=**, (Stu× W)-9 ×(AugE×IS)-1,**Line 4=**(Stu× W)-9 × (AugE×G)-16,**Line 5=**(Stu× W)-9× 58.15(AugE×J), **Line 6=**(Stu× W)-9 × (WT×AugE)-9,**Line 7=**(Stu× WT)-4 × (J×G)-1,**Line 8=**(AF× 12-1)-8 ×57.6(Aug×AugE), Tester 1=R.H-4 and Tester 2=HU-2

Table 6. Overall performance of L×T hybrids for major quantitative traits

Traits	Cross	SCA effect	GCA effect		Per se Performance	Combination
			Female	Male		
1.PHT	(1×2)	0.85*	-2.57**	-4.51**	48.77	H×L
	(3×2)	1.15**	-2.44**	-4.51**	49.20	H×L
	(7×1)	0.65*	-1.31**	4.51**	58.57	H×H
2. NOL	(2×1)	1.46*	-3.75**	-0.66**	25.10	H×H
	(3×2)	2.55**	-0.50ns	0.66**	30.77	H×L
	(6×1)	1.45*	0.27ns	-0.66**	29.10	L×H
3.LL	(5×1)	1.20**	1.20**	0.38**	12.37	H×H
4.LW	(2×2)	0.05*	0.21**	-0.15**	2.20	H×L
	(4×2)	0.05*	-0.09**	-0.15**	1.90	L×L
	(6×1)	0.05*	0.01ns	0.15**	2.30	L×H
	(7×2)	0.05*	0.01ns	-0.15**	2.00	H×L
	(8×1)	0.05*	0.01ns	0.15**	2.30	L×H
5.NOF	(1×1)	0.06*	-0.24**	0.09**	2.30	H×H
	(5×2)	0.09**	0.11**	-0.09**	2.50	H×L
	(7×1)	0.06*	0.26**	0.09**	2.80	H×H
6.DTF	(3×1)	-4.48**	-3.12**	-5.44**	89.53	H×H
	(4×2)	-1.75**	-1.22**	5.44**	105.03	L×L
	(5×2)	-2.07**	-1.24**	5.44**	104.70	H×L
	(7×2)	-1.27*	4.90**	5.44**	111.63	H×L
8.FLD	(2×1)	0.54*	0.10ns	0.57**	49.68	H×H
	(4×1)	0.53*	-0.47**	0.57**	49.10	L×H
9.BL	(4×1)	0.77**	0.10ns	0.41**	16.00	L×H
	(5×1)	0.60*	0.07ns	0.41**	15.80	H×H
	(6×2)	0.55*	0.82**	-0.41**	15.67	L×L

GCA=General combining ability,SCA=Specific combining ability, H×L=High×Low,……, L×L= Low×Low

On the basis of GCA effects out of 8 lines (DCF_1s) we can use line 5(P5), (Stu× W)-9 × 58.15 (AugE×J), line 2 (P2) (Stu× W)-9 ×57-6 (Aug×AugE) and line7 (P7), (Stu× WT)-4 × (J×G)-1 as mother line for seed production inside the plastic house. Likewise for homogenous seed production, among the tester, we can use tester1 (RH-4) as donor for those selected mother lines. Besides SCA effects indicated that none single crosses possessed all the traits under study. But some crosses demonstrated

significant SCA effects in positive direction for some traits. It is obvious that SCA effects indicated, it would not be possible to isolate crosses all traits are in the desirable combination. High specific combiner not only resulted from the combination of High×High general combiners but also obtained from the combination of High×Low and Low×Low general combiners. Narasimhamurthy and Gowda (2013) demonstrated same types of results in their research experiment.

Xuan et al. (2005) reported prevailing of additive type of gene action for the inheritance of quantitative traits viz. stem length, stem diameter, number of leaves, days to flowering, number of flowers, outer tepal length and attitude of the floral axis in *L.*× *formolongi*. But in this experiment, it is demonstrated that out of 10 growth and flowering traits so far we had studied some of them possessed dominance gene action. Stem diameter, number of leaves, leaf length, days to flowering, flower diameter and bud length indicated predominance of dominance gene action for the inheritance of these traits. While remaining traits like plant height, number of flowers, attitude of the floral axis (Xuan et al., 2005) and leaf length (Song et al., 2004) demonstrated additive type of gene action for the inheritance of these traits. Song et al. (2004) had also reported additive type of gene action for the inheritance of growth and flowering traits like plant height, length of leaves, the width of leaves, internode length, days to flowering and flower height etc.

CONCLUSIONS

The mean performance of all 26 genotypes indicated that improvement in some quantitative traits like days to flowering (DTF), bud length (BL) and attitude of the floral axis (AFA) can achieve as special L×T hybrids demonstrated superior performance for these traits as comparisons with lines (DCF_1s) and testers. We can exploit parent-5, (Stu× W)-9 × 58.15 (AugE×J) and Parent-2 (Stu× W)-9 ×57-6 (Aug×AugE) as mother lines and tester1 (RH-4) as donor line for commercial seed production system inside plastic house. In another hand, gene action clearly indicated that both types of gene action are important for the inheritance of studied growth and flowering traits. The additive type of gene action is found for acting for the inheritance of plant height, leaf length, the number of flower and attitude of the floral axis. While stem diameter, number of leaves, leaf length, days to flowering, flower diameter and bud length indicated prevailing of dominance gene action.

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