

LINE × TESTER ANALYSIS FOR AGRO-MORPHOLOGICAL TRAITS IN LINSEED (*Linum usitatissimum* L.)

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ABSTRACT

Fifteen linseed genotypes (*Linum usitatissimum* L.) were evaluated for combining ability through line × tester analysis using three diverse testers. A total of 63 entries (45 F₁'s + 18 parents) raised in randomized block design with three replications were evaluated for seed yield and related component traits. Parents and hybrids included in the investigation exhibited significant differences between treatments for all traits, indicating a considerable amount of variability. Both additive and non-additive gene actions exhibited significant roles in inheritance of seed yield and related traits with the preponderance of non-additive gene action due to higher magnitude of SCA variance over GCA variance. Based on combining ability studies, the lines viz., KL-310, KL-316 and K1 Raja were identified as best general combiners, whereas the cross combinations KL-312 × K 1 Raja, KL-313 × JRF-4 and KL-308 × K 1 Raja were found promising for seed yield and contributing traits. The highest value for heterobeltiosis and heterosis over standard check (Him Palam Alsi-2) was exhibited by KL-308 × JRF-4, KL-315 × Nagarkot, respectively.

Keywords: Combining ability, Gene action, Heterosis, Line × tester, Linseed

Oilseed crops are of pivotal significance in the Indian agricultural economy next to food grains with respect to area, production and value. Linseed (*Linum usitatissimum* L.) is one of the important non-edible oilseed crops and occupies a key position in oilseed production. Linseed or 'Alsi' is cultivated for seeds and fibers during the *rabi* season. Linseed fiber is used for making linen, while seed is used for oil and cake. Oil content in linseed varies from 33-45% (Thakur *et al.*, 2020) depending upon varieties.

India ranks fifth in linseed area and production globally but with low average productivity (605 kg/ha) compared to the global productivity 951 kg/ha (FAOSTAT 2020), which might be due to sub-marginal lands under unirrigated and *utera* conditions in different states of the country. Moreover, factors such as biotic stress, abiotic stress, limited resources and lack of improved varieties have lowered down the productivity of linseed (Rai *et al.*, 2002, Kumar and Paul 2015, Thakur *et al.*, 2020). But recently, it has gained an interest in the emerging market of functional food and manufacturing items of industrial significance due to the presence of essential polyunsaturated fatty acids such as omega-3 (36-57%) and omega-6 (18-24%) (Ganorkar and Jain 2013, Singh *et al.*, 2016, Nirala *et al.*, 2018, Ahmad *et al.*, 2021) which are not synthesized in the human body and must be ingested in food. Linseed oil has many beneficial effects on human health like reducing the

level of bad cholesterol, prevention of cardiovascular diseases, anti-inflammatory properties for rheumatoid arthritis, lignin an antioxidant for humans, along the manufacture of paints, varnish, linoleum, printing ink, pad-ink, oilcloth, patent leather, etc. (Singh *et al.*, 2016).

Conventional methods like selection and hybridization have generally been employed for genetic improvement of self-pollinated linseed crops. Genetic variability is a prerequisite for any breeding programme. The success of any hybridization programme relies on the choice of parents, breeding procedures, the ability of parents to combine, which depends upon complex interaction among the genes and their effects governing the expression of various traits. The parents exhibiting good *per se* performance may not always produce preferred cross combinations when used in hybridization programme (Allard 1960). Particular cross combination producing transgressive segregants would depend upon estimates of gene action involving additive, dominance, non-allelic interaction and linkage. Combining ability is the best tool to obtain information on the nature and magnitude of gene action while the heterosis to identify superior cross combinations produced by the parents. This study was conducted to evaluate the combining ability and heterosis, thus to generate new variability that could act as a repository for gene pool.

MATERIALS AND METHODS

Planting material and crossing design

The research work was conducted at experimental

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farm of Genetics and Plant Breeding, CSKHPKV, Palampur, H.P, situated at 1290.8 meters above mean sea level with annual rainfall varying from 1500 to 2500 mm. The soil is acidic with pH ranging from 5.0 to 5.6 and soil texture is silty clay loam. The material consisted of fifteen lines and three testers mated in line $\times$ tester fashion to produce 45 crosses during *rabi* 2018–2019. A total of 63 (18 parents + 45 F_1 's) entries were evaluated in RBD with three replications during *rabi* 2019–2020 with check Him Palam Alsi-2. In each replication, parents and F_1 's were grown in two row plot of 1 m length with a spacing of 30 cm $\times$ 10 cm. Recommended packages of practices were followed.

Data recording and statistical analysis

The data was recorded on five randomly selected competitive plants per plot across replications for primary branches/plant, secondary branches/plant, plant height (cm), technical height (cm), number of capsules/plant, seeds/capsule, seed yields (g), aerial biomass/plant (g), harvest index (%) and 1000-seed weight (g) whereas days to 50% flowering and days to 75% maturity were recorded on a plot basis. Analysis of variance was done as per Panse and Sukhatme (1985) and line $\times$ tester was done as per Kempthorne (1957). The data obtained were subjected to the statistical software TNAUSTAT (Manivannan, 2014) and further re-evaluated using OPSTAT (Sheoran *et al.*, 1998).

Per cent contribution of lines, testers, and their interactions were computed as per the formulae suggested by Singh and Chaudhary (1985):

Per cent contribution of lines = $SS(\text{lines})/SS(\text{crosses}) \times 100$; Per cent contribution of testers = $SS(\text{testers})/SS(\text{crosses}) \times 100$; Per cent contribution of lines $\times$ testers = $SS(\text{lines} \times \text{testers})/SS(\text{crosses}) \times 100$ where: SS = sum of squares.

Heritability in the narrow sense (h_{ns}^2) was calculated according to Singh and Chaudhary (1985):

$$\text{Heritability in the narrow sense } (h_{ns}^2) = \frac{\sigma^2_A}{\sigma^2_p}$$

σ^2_A = Additive variance; σ^2_p = Phenotypic variance

The magnitude of heritability was classified as high (>50%), moderate (30%-50%) and low (<30%) as per Bhateria *et al.* (2006).

Heterosis was estimated as per Fonseca and Patterson (1968).

$$\text{Heterosis (\%)} \text{ over better parent (H1)} = \frac{\bar{F}_1 - \overline{BP}}{\overline{BP}} \times 100$$

$$\text{Heterosis (\%)} \text{ over standard check (H2)} = \frac{\bar{F}_1 - \overline{SC}}{\overline{SC}} \times 100$$

where,

$\bar{F}_1$ = Mean of F_1 's

$\overline{BP}$ = Mean of better parent

$\overline{SC}$ = Mean of standard check

RESULTS AND DISCUSSION

Analysis of variance for combining ability effects

ANOVA for yield and related traits revealed that both lines and testers exhibited significant differences ($P \leq 0.05$) for all traits except days to 75% maturity and seeds per capsule in case of lines while primary branches per plant, seeds per capsule and aerial biomass per plant in case of testers. The mean square values due to line $\times$ tester were significant for most of the traits except days to 50% flowering, 75% maturity and technical height, indicating considerable genetic variability for specific combining ability among crosses (Table 2). Mean sum of square values represents significant variation among crosses for combining ability hence, allows estimates of GCA and SCA effects as reported earlier by Pali and Mehta (2014), Bisht and Paul (2019), Upadhyay *et al.* (2019), Mahawar *et al.* (2021). The significance of GCA effects of lines and testers for different traits revealed additive gene effects, hence selection of promising parents, which are good combiners for these traits could be used for hybridization programmes while significant SCA effects indicate non-additive gene effects, which clearly means that both additive and non-additive gene actions contribute towards variability. Parents were categorized as good, average and poor based on general combining ability performances. The parental genotypes that exhibited significant GCA value in the desired direction and in opposite direction, were termed as good and poor combiners, respectively. In contrast, parents with non-significant GCA value in either direction were average combiners. Though none of the parents were found to be good combiners for all traits. However, lines viz., KL-308, KL-310 and KL-316 were good general combiners for primary branches, secondary branches, plant height, technical height, seed yield per plant and 1000-seed weight. Among testers, JRF-4 and K 1 Raja could be selected as parents for days to 50% flowering and secondary branches per plant, plant height, technical height, seed yield per plant and harvest index, respectively (Table 3). Four cross combinations viz., KL-318 $\times$ JRF-4, KL-306 $\times$ JRF-4, KL-313 $\times$ JRF-4, and KL-312 $\times$ K 1 Raja, were accounted to be specific combiners for seed yield and related component traits. Based on GCA effects of parents and high SCA effects, no specific trend was observed which is in accordance with Bhateria *et al.* (2006) and Bisht and Paul (2019) indicating that in

Table 1. List of parental genotypes and their parentage/source

Line	Parentage/Source	Line	Parentage/Source
KL-305	TL-27 × Nagarkot	KL-315	TL- 27 × Flak-1
KL-306	Nagarkot × T-397	KL-316	Him Alsi-2 × Binwa
KL-307	Him Alsi-2 × Nagarkot	KL-317	Him Alsi-1 × Binwa
KL-308	T-397 × Nagarkot	KL-318	Him Alsi-2 × TL-11
KL-309	Canada × Nagarkot	KL-319	(KL-243 × Janki) × KL-243
KL-310	Giza-8 × Nagarkot	Testers	
KL-311	Giza-6 × Nagarkot	K 1 Raja	CSIRO, Canberra, Australia
KL-312	Giza-7 × Nagarkot	Nagarkot	New River × LC-216
KL-313	Faiking × Nagarkot	JRF-4	CRIJAF, Barrackpore
KL-314	Belinka-60 × Nagarkot		

[CSIRO: Commonwealth Scientific and Industrial Research Organization, CRIJAF: Central Research Institute for Jute and Allied Fibers]



Fig. 1. Parents showing flower morphology

general, the general combining ability had no bearing on the SCA effects of the crosses.

The presence of high SCA effects (plant height) in the desired direction accompanied by good × good general combiners (KL-317 × K 1 Raja) is due to the

presence of fixable higher order additive interactions. When good × poor general combiners (KL-312 × K 1 Raja) result in the manifestation of high SCA effects (seed yield per plant) it relates to favorable additive effects of good general combiner and epistatic effects of

Table 2. Analysis of variance for combining ability effects for various traits in linseed

Sources	Lines	Testers	Lines × Testers	Error
Df	14	2	28	88
Character				
Days to 50% flowering	69.54**	198.29**	29.53	22.58
Days to 75% maturity	10.73	38.94*	8.65	8.95
Primary branches per plant	9.83**	0.61	2.98**	0.41
Secondary branches per plant	10.47**	6.44**	6.01**	1.01
Plant height (cm)	150.66**	1786.06**	92.01**	10.34
Technical height (cm)	123.61**	1617.22**	33.31	24.77
Capsules per plant	541.39**	231.12**	517.89**	21.56
Seeds per capsule	0.38	0.10	0.47*	0.26
Seed yield per plant (g)	7.52**	33.30**	6.93**	0.37
Aerial biomass per plant (g)	1264.51**	16.93	484.59**	45.23
Harvest index (%)	9.84**	93.47**	16.92**	2.14
1000-seed weight (g)	3.55**	0.43**	0.95**	0.04

* Significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$

poor general combiner, which supplemented desirable plant attributes as in accordance with Bhateria (2006), Nirala *et al.* (2018), Bisht and Paul (2019). Population improvement, modified pedigree breeding programme, heterosis breeding and random mating along with a selection among segregants (i.e. recurrent selection) may be utilized to harness both additive and non-additive gene effects. Epistatic gene action (non-allelic interaction) dominance × dominance type is especially responsible for higher magnitude of SCA effects when poor × poor general combiner parents interact with each other. This type of non-fixable gene action can be fixed by breeding methods such as biparental mating or reciprocal recurrent selection followed by pedigree method which can be used for the genetic improvement for various yield related characters in linseed. Similar results were earlier reported by Bhateria (2006), Kumar and Paul (2015), Singh *et al.* (2016), Bisht and Paul (2019) in linseed.

Analysis of heterosis

Heterosis is an effective tool to identify superior F_1 over the mid or better parent or best check. Assessing the heterotic effects in the crosses derived from genetically diverse parents, in terms of yield, its various attributing traits had been always an objective of an experiment. Heterosis may be in the positive direction as in case of most of the quantitative traits related to yield or in the negative direction desirable for earliness and short height plants in linseed. The results indicated that heterosis over better parent/heterobeltiosis was exhibited by KL-313 × JRF-4 and KL-306 × JRF-4 for seed yield traits. However, for standard heterosis (Him

Palam Alsi-2) none of the cross combination were superior to Him Palam Alsi-2 for all traits, nevertheless, eleven cross combinations (Table 4) exhibited superiority for eight quantitative traits. In case of heterobeltiosis the maximum value was attained by seed yield per plant (KL-308 × JRF-4, 179.75%) followed by capsules per plant (KL-312 × Nagarkot, 168.49%) and aerial biomass per plant (KL-308 × Nagarkot, 149.68%) while, in case of standard heterosis maximum value was observed for seed yield per plant (KL-315 × Nagarkot, 218.27%) followed by aerial biomass per plant (KL-308 × Nagarkot, 195.71%) and capsules per plant (KL-308 × K 1 Raja, 170.16%). These results agreed with the earlier results of Pali and Mehta (2014), Chaure *et al.* (2018), Dhirhi *et al.* (2018). The observed high heterotic effects might be useful in heterosis breeding or for the production of superior lines, which is difficult to isolate in a small population of heterotic crosses in which the number of segregating loci with additive gene effect is relatively large. So, it would be better to adopt the biparental cross approach for accumulating favorable and additive gene effects as it is expected to offer maximum promise in breeding for high yield along with the identification of cross combinations throwing superior segregants using heterosis breeding for linseed improvement.

Estimation of genetic components

The estimates of genetic components of variance (Table 5) revealed higher magnitude of σ^2_{SCA} than σ^2_{GCA} for all the characters, indicating the major role played by non-additive gene action for the traits marked by higher magnitude of dominance variance than additive variance which is further confirmed by

Table 3. Top ranking parents and cross combinations based on their GCA and SCA estimates

Character	Parents	GCA effect	Crosses	SCA effect	GCA effects of parents
DF	KL-310	-3.64*	KL-318 × Nagarkot	-6.47*	Poor × Average
	KL-307	-3.31*	-	-	-
	KL-305	-3.20*	-	-	-
DM	JRF-4	-1.07*	-	-	-
PB	KL-308	1.52**	KL-318 × JRF-4	1.14**	Average × Poor
	KL-310	1.44**	KL-316 × Nagarkot	1.10**	Average × Average
	KL-309	0.94**	KL-317 × Nagarkot	1.10**	Average × Average
SB	KL-308	2.07**	KL-308 × K 1 Raja	2.37**	Good × Good
	KL-316	1.64**	KL-306 × JRF-4	2.17**	Poor × Poor
	KL-310	0.92**	KL-313 × JRF-4	1.78**	Average × Poor
PH (cm)	K 1 Raja	7.10**	KL-313 × JRF-4	9.11**	Poor × Poor
	KL-316	6.35**	KL-317 × K 1 Raja	7.77**	Good × Good
	KL-308	6.28**	KL-314 × K 1 Raja	7.67**	Poor × Good
TH (cm)	K 1 Raja	6.92**	KL-306 × K 1 Raja	8.05**	Good × Good
	KL-306	5.04**	KL-318 × JRF-4	5.96*	Poor × Poor
	KL-311	4.91**	-	-	-
C/P	KL-308	12.82**	KL-308 × K 1 Raja	26.51**	Good × Average
	KL-312	12.09**	KL-313 × JRF-4	21.91**	Good × Average
	KL-316	5.47**	KL-315 × JRF-4	17.41**	Poor × Average
S/C	KL-305	0.37*	KL-319 × JRF-4	0.72*	Average × Poor
	-	-	KL-312 × K 1 Raja	0.63*	Average × Average
SY/P(g)	KL-308	1.88**	KL-312 × K 1 Raja	2.98**	Poor × Good
	KL-313	1.40**	KL-305 × Nagarkot	2.17**	Poor × Good
	KL-306	1.04**	KL-311 × JRF-4	2.13**	Poor × Poor
AB/P (g)	KL-308	31.70**	KL-319 × Nagarkot	9.45*	Poor × Poor
	KL-313	17.98**	KL-311 × JRF-4	15.71**	Poor × Average
	KL-316	7.29**	KL-312 × K 1 Raja	13.55**	Poor × Average
HI (%)	KL-310	1.92**	KL-319 × K 1 Raja	5.79**	Average × Good
	Nagarkot	1.18**	KL-310 × K 1 Raja	3.10**	Good × Good
	KL-306	1.14*	KL-318 × JRF-4	2.64**	Average × Poor
1000-SW (g)	KL-315	1.06**	KL-314 × Nagarkot	0.94**	Average × Good
	KL-307	0.85**	KL-317 × Nagarkot	0.82**	Poor × Good
	KL-308	0.71**	KL-316 × K1 Raja	0.76**	Good × Poor

* Significant at $P \leq 0.05$ **Significant at $P \leq 0.01$

σ^2A/σ^2D ratio, where all the traits have values less than unity and assisted by the results obtained from average degree of dominance that showed the presence of over dominance (>1) for the inheritance of characters under study. Similar results were reported by Kumar and Paul (2015) for plant height, primary branches, secondary branches and capsules per plant; Rastogi and Shukla (2018) observed it for primary branches per plant, the number of capsules per plant, the number of seeds per capsule and seed yield per plant; Bisht and Paul (2019)

for all the traits; Upadhyay *et al.* (2019) for secondary branches per plant, plant height and technical height, reported the predominance of non-additive gene action. Whereas, Yadav *et al.* (2013), Magar *et al.* (2019) favored the importance of both non-additive and additive gene action for most of the traits.

The narrow sense heritability (h^2_{ns}) estimates showed low values for all the traits indicating that a non-fixable component of variation was governing these characters. Bhateria *et al.* (2006) also reported

Table 4. Top ranking cross combinations based on heterobeltiosis (%) and standard heterosis (%) for different characteristics

Character	Superior cross over better parent	H1 (%)	Superior cross over standard check	H2 (%)
Primary branches per plant	KL-308 × K 1 Raja	82.67	KL-308 × K 1 Raja	96.28
	KL-317 × Nagarkot	78.84	KL-310 × K 1 Raja	84.04
	KL-308 × Nagarkot	70.30	KL-308 × Nagarkot	82.98
Secondary branches per plant	KL-308 × K 1 Raja	97.83	KL-308 × K 1 Raja	106.82
	KL-305 × Nagarkot	88.66	KL-316 × K 1 Raja	87.54
	KL-308 × Nagarkot	64.71	KL-313 × JRF-4	76.14
Plant height (cm)	KL-313 × JRF-4	8.87	KL-317 × K 1 Raja	43.52
	KL-306 × JRF-4	7.87	KL-316 × K 1 Raja	39.59
	-	-	KL-308 × K 1 Raja	38.67
Technical height (cm)	-	-	KL-306 × K 1 Raja	54.92
	-	-	KL-311 × K 1 Raja	38.07
	-	-	KL-316 × K 1 Raja	37.20
Capsules per plant	KL-312 × Nagarkot	168.49	KL-308 × K 1 Raja	170.16
	KL-308 × K1 Raja	155.17	KL-313 × JRF-4	138.33
	KL-317 × Nagarkot	115.20	KL-312 × Nagarkot	132.02
Seed yield per plant (g)	KL-308 × JRF-4	179.75	KL-315 × Nagarkot	218.27
	KL-313 × JRF-4	169.42	KL-308 × Nagarkot	203.24
	KL-306 × JRF-4	168.79	KL-312 × K 1 Raja	200.79
Aerial biomass per plant (g)	KL-308 × Nagarkot	149.68	KL-308 × Nagarkot	195.71
	KL-319 × Nagarkot	133.17	KL-308 × K 1 Raja	189.41
	KL-313 × Nagarkot	120.20	KL-308 × JRF-4	160.93
Harvest index (%)	KL-319 × K1 Raja	137.16	KL-319 × K1 Raja	87.1
	KL-315 × K1 Raja	130.76	KL-310 × K 1 Raja	74.16
	KL-309 × K1 Raja	114.79	KL- 307 × Nagarkot	60.37
1000-seed weight (g)	KL-319 × JRF-4	23.06	KL-315 × Nagarkot	49.84
	KL-313 × JRF-4	18.70	KL- 307 × Nagarkot	41.01
	KL-310 × K1 Raja	18.18	KL-314 × Nagarkot	38.16

[H1: Heterobeltiosis, H2: Standard heterosis]

low estimates of narrow sense heritability for primary branches per plant, capsules per plant, seeds per capsule, seed yield per plant and harvest index while low estimates for all traits were observed by Bisht and Paul (2019) representing the dominance of non-fixable components of variation for all the traits indicating that the direct selection will be ineffective but selection from the segregating generations derived from such crosses will be effective. Low heritability would also enable the use of heterosis which could be achieved by crossing divergent lines like in biparental mating.

The proportional contribution of lines, testers and line × tester interactions towards the total sum of squares ranged from 17.25% to 64.48%, 0.11% to 54.84% and 15.81% to 70.46%, respectively. The

percent contribution of lines was higher than testers and line × tester interaction for the characteristics such as days to 50% flowering, primary branches per plant, aerial biomass per plant and 1000-seed weight. While, for days to 75% maturity, secondary branches per plant, capsules per plant, seeds per capsule, seed yield per plant and harvest index, line × tester interaction was higher. Testers contributed more towards the sum of squares due to plant height and technical height. From Table 5 it can be concluded that, line × tester interaction played a significant role in the expression of most of the characters, indicating the higher estimates of variances due to specific combining ability that further confirmed the active involvement of non-additive gene action in the parameters studied. The contribution of lines towards

Table 5. Estimates of genetic components for various traits in linseed

Character	$\sigma^2\text{GCA}$	$\sigma^2\text{SCA}$	Additive variance $\sigma^2\text{A}$	Non- additive variance $\sigma^2\text{D}$	$\sigma^2\text{A}/\sigma^2\text{D}$	Average degree of dominance $\sqrt{\sigma^2\text{D}/\sigma^2\text{A}}$	Herita- bility (%) h^2_{ns}	Contribution (%)		
			F = 1	F = 1				Lines	Testers	Interac- tion
DF	0.260	2.317	0.520	2.317	0.22	2.11	1.40	44.32	18.05	37.63
DM	0.026	-0.100	0.052	0.100	0.52	1.39	0.61	31.94	16.56	51.49
PB	0.026	0.856	0.053	0.856	0.06	4.01	1.24	61.89	0.55	37.56
SB	0.018	1.669	0.037	1.669	0.02	6.72	0.51	44.72	3.93	51.36
PH (cm)	1.218	27.222	2.436	27.222	0.09	3.34	2.10	25.54	43.26	31.2
TH (cm)	1.282	2.845	2.565	2.845	0.90	1.05	3.20	29.34	54.84	15.81
C/P	-0.071	165.446	0.142	165.446	0.00	34.13	0.05	33.62	2.05	64.33
S/C	-0.001	0.072	0.001	0.072	0.01	8.49	0.35	28.49	1.05	70.46
SY/P (g)	0.018	2.187	0.035	2.187	0.02	7.90	0.51	28.77	18.21	53.03
AB/P (g)	2.889	146.455	5.778	146.455	0.04	5.03	1.51	56.55	0.11	43.34
HI (%)	0.016	4.929	0.031	4.929	0.01	12.61	0.31	17.25	23.41	59.34
1000-SW (g)	0.010	0.304	0.021	0.304	0.07	3.80	2.40	64.48	1.11	34.41

variation followed the line $\times$ tester interaction, whereas, tester's contribution seems negligible for most of the characters.

To conclude, the combining ability analysis helped in identifying parents KL-308, KL-310 and KL-316 for crossing programme resulting in superior genotypes with higher seed yield and cross combinations KL-318 $\times$ JRF-4, KL- 306 $\times$ JRF-4, KL-313 $\times$ JRF-4 and KL-312 $\times$ K 1 Raja were identified as potential crosses for exploitation through heterosis breeding, providing that the technical problems hampering economical production of hybrid seeds in linseed could be overcome.

Authors' contribution

Conceptualization of research work and designing of experiments (SP, RT); Execution of field/lab experiments and data collection (RT); Analysis of data and interpretation of results (RT, SP); Preparation of manuscript (RT, SP).

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