

## Research Article



## Combining Ability for Seed Yield in Indigenous and Exotic *Brassica napus* Genotypes

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**Abstract** | Combining ability was estimated for seed yield using biplot approach in fifteen parental genotypes following 11× 4: line × tester fashion. The resultant 44 F<sub>1</sub> hybrids along with parental genotypes were evaluated under field condition in a replicated trial for seed yield plant<sup>-1</sup> performance. Differences among the parental lines, testers, F<sub>1</sub> hybrids and line × tester interaction for found significant (P<0.01) for the studied parameter. Promising genotypes for seed yield *i.e.* L-4, L-5, L-7 and T-4 were identified as best parents as they outclassed all other genotypes by producing maximum seed yield per plant of 28 g. Since, the line × tester source was significant, hence data was further subjected to combining ability analysis. For the inheritance of the studied trait (seed yield plant<sup>-1</sup>), additive as well as non-additive genetic mechanisms were found important due to significant effects for both components *i.e.* General Combining Ability (GCA) and Specific Combining Ability (SCA) effects. Maximum GCA was shown by L-7 and T-1; whereas high SCA was noted for L-6 and L-7 with good ability to combine with T-3 and T-1 respectively, and could be utilized as genetic material in future breeding programs. Overall the biplot approach was found much cooperative in elaborating the genetic parameters of the data on the basis of its high explanatory aptitude and graphical demonstration.

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### Introduction

Oilseed rape (*Brassica napus* L.) is one of the most essential oil crops. At present it is one of the largest sources of vegetable oil. It ranked third all over the world after Palm and Soybean oil (Miri, 2007). In Pakistan, most of the local oil is extracted from cotton seed but is not a traditional oil seed crop. Other crops like sunflower and maize are also cultivated for oil purposes. Among the traditional crops, brassica is the major contributor (Ali and Mirza, 2005). Brassica is mostly planted on marginal lands dispersed in the whole country on substantial area. The requirement for edible oil in the country is increasing every year

due to increase in population. Which in turn increases the per capita consumption of edible oil. Total edible oil requirement of the country during 2010-11 was 2.5 million tons while domestic production was only 0.6 million tons from all oilseed sources, whereas the remaining was imported. Every year a huge amount (about 2 billion of US \$) is being spent on this import to overcome the shortage of edible oil in the country. In Pakistan, total cultivated area under brassica was 233 thousand hectares producing 188 thousand tons seed with a seed yield of 800 kg ha<sup>-1</sup> (Anonymous, 2013). In the Khyber Pakhtunkhwa province, the total area under brassica was 18 thousand hectares producing 7.4 thousand tons seed with an average seed yield of 435 kg ha<sup>-1</sup>.

Development of superior varieties to improve the seed yield has always been one of the major plans in crop breeding programs. Rapeseed breeding events are mainly designed with the objectives to evolve new genotypes with persistent high seed yield and quality oil. High seed yield is one of the prime objectives in rapeseed breeding program. Seed yield trait is quantitative in nature and is highly subjective to the genes involve in its inheritance. Moreover, environmental factors and their interaction with the genotype is also influencing this trait (Huhn and Leon, 1985). Restructuring the genes through artificial hybridization from suitable parents could produce superior varieties. It is also required to have considerable knowledge about the nature of gene action accountable for governing a trait under improvement. Moreover, general combining ability of the parental genotypes and specific combining ability of their hybrid combinations also provide initial valuable information about genotypes for their manipulation in future crop breeding program. Both combining ability effects (*i.e.* GCA and SCA) are used as main indicators for the identification and selection of potential inbred lines in most of crop breeding program for the development of hybrid and open pollinated cultivars. Among others techniques, the (line  $\times$  tester) approach is used as an effective methods for evaluation of large number of inbred lines based on their ability to combine with each other. In addition, this methodology also delivers evidence on the comparative position of general combining ability and specific combining ability effects for understanding the genetic architecture of various important plant attributes (Singh and Chaudhary, 1985).

Conventional combining ability analysis (Griffing, 1956) split the total genetic variation into two main components *i.e.* general combining ability of the parental genotypes and specific combining ability of the crosses. However, a new biplot approach (Yan and Hunt, 2002) has been developed for analyzing the data regarding combining abilities, heterosis and relationships among parents. This new approach provides a graphical demonstration of the data using principle components (PC<sub>1</sub> and PC<sub>2</sub>) which are obtained through principle component analysis (Yan and Hunt, 2002). Combining ability via biplot has been previously reported in maize (Khalil et al., 2010). Keeping in view the importance of combining ability and heterosis in plant breeding, the present study was planned with the objectives (i) to study general

combining ability and specific combining ability of the parents and crosses respectively, and (ii) to find out best combiners for future rapeseed breeding programs.

## Materials and Method

### Experimental site

The present study was conducted at the research farm of the University of Agriculture Peshawar-Pakistan.

### Genetic material

Genetic material for this experiment comprised of a set of 15 *Brassica napus* genotypes, each with distinct genetic background. Eleven out of 15 genotypes (L-1 to L-11) introduced from China were used as lines (double haploid) and the remaining four genotypes (T-1 to T-4) procured from National Agriculture Research Centre, Islamabad were commercial cultivars (Open pollinated varieties) used as testers.

**Development of F<sub>1</sub> hybrids:** During the first growing season, all the 11 parental introduced genotypes (lines) were manually crossed with four commercial genotypes (testers) in a line  $\times$  tester fashion to produce 44 F<sub>1</sub> hybrids. Crosses were made using manual emasculation and pollination. For emasculation, young buds were selected and the petal whorl was removed with the help of forceps in order to expose the anthers. Unripe anthers were removed with the forceps. To avoid contamination by foreign pollens, the emasculated buds were sheltered with butter paper bags. The branches with emasculated buds were labeled with the genotype name, date of emasculation and other necessary information. The next day fresh flowers from the male parents were selected and used to pollinate the emasculated buds of the female parental genotypes. After shedding pollens, the buds were again covered with the same butter paper bags to avoid contamination with unknown pollens. At maturity all the pods for each cross combination were harvested, properly sundried, threshed and stored for use in next generation.

**Evaluation of F<sub>1</sub> hybrids and data recording:** The resultant F<sub>1</sub> hybrids were evaluated along with parental genotypes in a randomized complete block (RCB) design at the University of Agriculture Peshawar, Pakistan. Each plot was comprised of two rows having five meter length. Row to row distance was maintained as 50 cm whereas plants within a row were kept at

15 cm distance. Normal cultural practices were used throughout the crop growing period. Data for seed yield was recorded on individual plant basis for which a randomly selected 15 plants from each plot in each replication investigated.

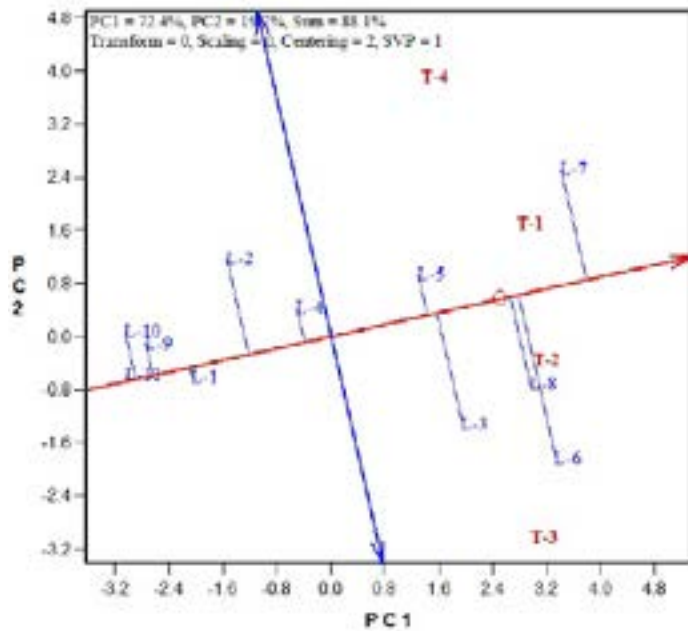


Figure 1: Biplot based on seed yield data explaining combining ability in brassica genotypes

Data Analysis

**Analysis of variance:** Data recorded for seed yield plant<sup>-1</sup> was statistically analyzed according to the procedure for line by tester as narrated by Singh and Chaudhary (1985).

**Biplot analysis for combining ability:** Following analysis of variance the data were subjected to biplot analysis according to the method of (Yan and Hunt, 2002) and (Bertoia et al., 2006). GGE biplot methodology for combining abilities (GCA and SCA) in a line × tester data set was used, with the following model as:

$$Y_{ij} - \beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \epsilon_{ij}$$

Where:

$Y_{ij}$ : genotypic value of the cross between i<sup>th</sup> line and j<sup>th</sup> tester;  $\beta_j$ : average value for crosses involving j<sup>th</sup> tester;  $\lambda_1$ : singular value for PC<sub>1</sub>;  $\lambda_2$ : singular values for and PC<sub>2</sub>;  $\xi_{i1}$  and  $\eta_{j1}$ : eigenvectors for PC<sub>1</sub> associated with i<sup>th</sup> line;  $\xi_{i2}$  and  $\eta_{j2}$ : eigenvectors for PC<sub>2</sub> associated with j<sup>th</sup> tester;  $\epsilon_{ij}$ : overall residual of the model associated with the combination of line i and tester j.

Symmetrical scaling was carried out for Principal  
March 2017 | Volume 33 | Issue 1 | Page 179

components scores for entries and testers (Yan and Hunt, 2002; Bertoia et al., 2006). The analyses reported in this study were performed with the GGE-biplot software. Which is a window based application that generates biplots for a two-way data set (Yan, 2001).

Table 1: Mean squares for seed yield plant<sup>-1</sup> in parents and F<sub>1</sub> crosses evaluated during 2011-12.

Source of variance	df	Seed yield plant <sup>-1</sup> (g)	
		Mean Squares	% of SS
Replication	2	0.6	-
Genotype	58	192.0**	-
Parents	14	98.3**	-
F <sub>1</sub> s	43	216.6**	83.7
parents vs F <sub>1</sub> s	1	444.4**	-
Lines	10	540.7**	58.0
Testers	3	470.0**	15.1
L × T	30	83.3**	26.8
Error	116	1.7	-

\*, \*\*: Significant at 5 and 1% level of Probability, respectively

Results and Discussion

The results obtained from the analysis of variance and biplot Figures 1, 2 and 3 of the studied trait are given as following to describe various features of the experimental material.

Results of the data concerning seed yield plant<sup>-1</sup> clearly demonstrated variation (P<0.01) among the genotypes under investigation. The genotype main effect was further partitioned into effects for parents and F<sub>1</sub> hybrids which were also found significant. In line by tester ANOVA (analysis of variance), the variances for lines, testers and interaction (L × T) effect were also found significant (P<0.01) (Table 1). These significant effects suggested huge diversity among selected parents and their capability to transfer this into their hybrids. Seed yield is the outcome of different yield associated components. The crop species cultivated for their utilizable seeds are always desired to have cultivars with a potential of producing high seed yield. Among the parental lines, L-5 attained maximum seed yield plant<sup>-1</sup> (28.2 g) and remain statistically at par with T-4 (28.0 g), L-7 (27.7 g) and L-4 (27.6 g). Low yielding line was L-11 which attained minimum 12.7 g of seed yield plant<sup>-1</sup> (Table 2). Likewise, among the hybrids highest seed yield plant<sup>-1</sup> of 43.9 g was recorded for L-6 × T-3 followed by L-8 ×

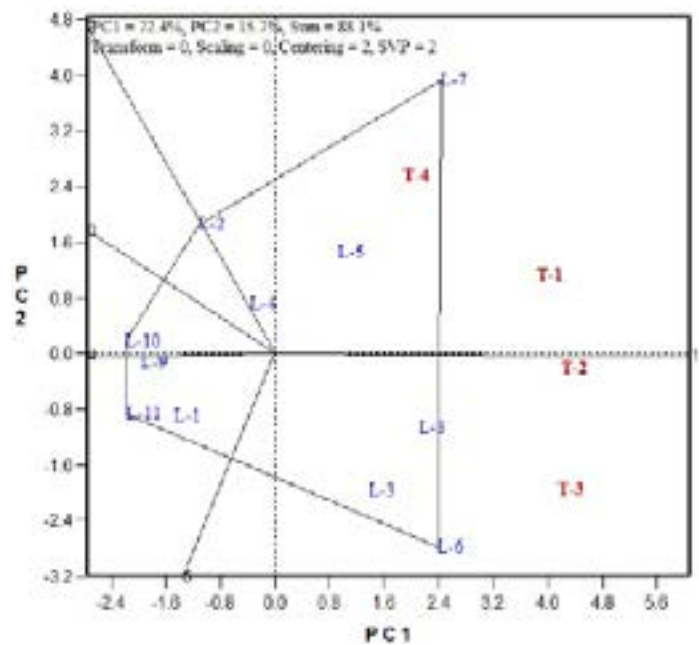


T-3 (40.0 g) and L-7 × T-1 (38.5 g). Minimum seed yield plant<sup>-1</sup> was observed for four hybrids i.e. L-9 × T-2 (10.8 g), L-2 × T-2 (11.0 g), L-10 × T-2 (11.3 g) and L-11 × T-2 (11.5 g) and they remain statistically at par (Table 3). The cross combinations that yielded high quantity of seeds plant<sup>-1</sup> were interesting due to the involvement of parents that were even not high yielding themselves. This phenomenon is further explained under the following section of combining ability analysis.

**Table 2: Mean values for seed yield of parental genotypes evaluated during 2011-12**

Genotypes	Code name	Seed yield plant <sup>-1</sup> (g)
AUP-401	L-1	15.7
AUP-402	L-2	20.9
AUP-403	L-3	24.1
AUP-404	L-4	27.6
AUP-405	L-5	28.2
AUP-406	L-6	20.1
AUP-407	L-7	27.7
AUP-408	L-8	20.3
AUP-409	L-9	15.3
AUP-410	L-10	14.3
AUP-411	L-11	12.7
Mean of Lines	-	20.6
Concord	T-1	16.3
Ac-elect	T-2	12.7
Shiralee	T-3	21.0
Hoyla-43	T-4	28.0
Mean of Testers	-	19.5
LSD <sub>0.05</sub>	-	1.2

The biplot regarding combining ability (GCA and SCA) of the genotypes for the trait under study explained 88.1% of the total variation, in such a way that both components (PC<sub>1</sub> and PC<sub>2</sub>) explained 72.4 and 15.7% variation, respectively. The projection of the markers of parental genotypes onto ATC x-axis depicted significant GCA effects. Among the female parents (lines) L-7 was identified as best general combiner, followed by L-6, L-8, L-3 and L-5. Maximum GCA effects of these parental lines were confirmed by their positive interaction with all the testers. Poor general combining ability was exhibited by remaining six lines i.e. L-11, L-10, L-9, L-1, L-2 and L-4. Interaction of these poor general combiners was negative with all the testers. Similarly, among the testers T-1 was identified as best general combiner being highly discriminating and representative (Figure 1). The polygon



**Figure 2: Biplot based on seed yield data explaining specific cross combination in brassica genotypes**

view of the biplot (Figure 2) identified best hybrid combinations. Among the lines, L-6 and L-7 were identified as best specific combiners showing their potential to produce superior and heterotic crosses especially with T-3 and T-1, respectively. The worst specific combiners were L-11, L-10 and L-9, as discussed earlier none of these lines could interact positively with any of the testers. The projections of parental genotypes onto the ATC x-axis and y-axis varied significantly which suggested that both general combining ability (GCA) and specific combining ability (SCA) effects contributed in the expression of seed yield per plant trait. Since, GCA and SCA provides estimates for additive and non-additive genetic effects therefore, it can be concluded that both gene actions (additive and non-additive) were involved in the inheritance of seed yield per plant trait. However, the magnitude of GCA was comparatively more than the magnitude of SCA which signified the role of additive type of gene action for seed yield per plant in this set of genotypes. Rameeh (2012) also reported that both general and specific combining ability effects contributed in the expression of yield and its associated components in spring type of Brassica. Similarly, Huang et al. (2010) and Sabaghnia et al. (2010) reported that general and specific combining abilities (GCA and SCA) effects were responsible for expression of seed yield trait in winter type of rapeseed. These researchers noted that non-additive genetic effects were more important for yield associated traits which might be due to genetic differences in genotypes used in the present study and their studies.

**Table 3:** Mean values for seed yield plant<sup>-1</sup> of F<sub>1</sub> hybrids evaluated during 2011-12

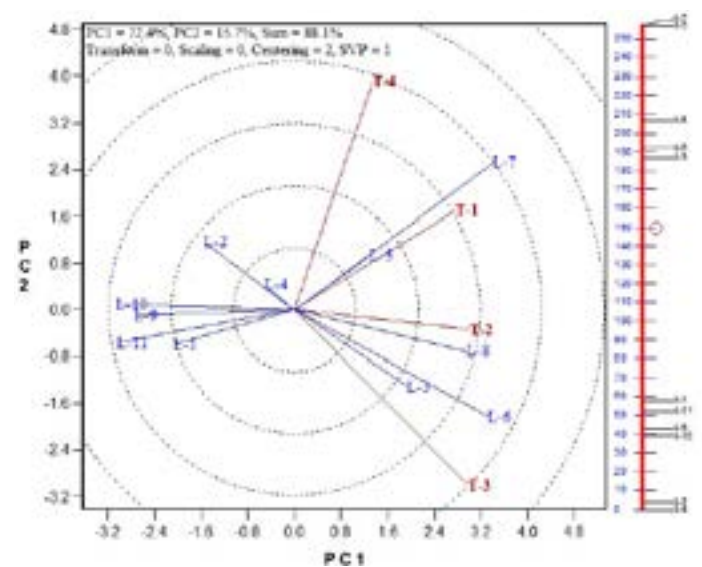
Lines	Seed yield plant <sup>-1</sup> (g)				
	Testers				Mean
	T-1	T-2	T-3	T-4	
L-1	14.7	14.2	23.2	21.8	18.5
L-2	17.0	11.0	23.7	33.4	21.3
L-3	22.2	31.3	34.7	24.0	28.1
L-4	24.3	18.3	22.9	25.0	22.6
L-5	23.7	18.7	34.0	37.4	28.5
L-6	30.4	27.4	43.9	23.5	31.3
L-7	38.5	32.5	25.9	37.1	33.5
L-8	30.1	26.1	40.0	28.3	31.1
L-9	15.8	10.8	19.3	21.7	16.9
L-10	15.3	11.3	16.7	21.2	16.1
L-11	14.5	11.5	18.7	18.4	15.8
Mean	22.4	19.4	27.5	26.5	

LSD<sub>0.05</sub> value for F<sub>1</sub> hybrids: 1.21

Moreover, the percent variation explained by lines and testers collectively (58.0+15.1% = 73.1%) is much larger than 26.8% explained by L × T also confirmed that additive genetic control mechanism was more important as compared to non-additive for seed yield per plant in the present study. Similar findings were reported by Farshadfar et al. (2013) who were of the opinion that additive type of gene action was more important in rapeseed line by tester crosses. Huang et al. (2009) suggested that additive genetic effects were predominant in controlling seed yield per plant in brassica. The results reported by Ghosh et al. (2002) revealed that, both additive and dominance type of gene actions were involved in controlling seed yield per plant trait in Indian mustard. In contrast, Cheema and Sadaqat (2004) found that non-additive genetic mechanism is involved in the inheritance of seed yield per plant in brassica. It is important to mention that the significant non-additive portion of genetic variance is also an important component which can be utilized in heterosis breeding. High heterotic responses coupled with positive significant general and specific combining ability effects were considered as important factors for enhancing seed yield per plant in brassica (Sincik et al., 2011). This phenomenon is also explained by Kadkol et al. (1984) that high heterosis of a hybrid is mostly dependent on the combining abilities of its parental genotypes.

The entry-tester relationship biplot of the seed yield plant<sup>-1</sup> explained the genetic relations of the testers

in such a way that the vectors of T-4 and T-3 were considerably distant from each other while T-1 and T-2 were placed in the middle of both in a way that T-2 was more related to T-3 and T-1 to T-4. The female parents (lines) were grouped into two well defined clusters i.e. [L-1,L-11,L-9,L-10,L-2,L-4] and [L-7,L-5,L-8,L-6,L-3] (Figure 3). This relationship of the lines can also be confirmed from the average tester coordination view of the biplot, where all the lines of the first cluster were weak general combiners and interacted negatively with all the testers, whereas the lines of the second cluster were good general combiners and also interacted positively with all the testers (Figure 2). This relation pointed towards the involvement of common gene(s) in the genotypes within a cluster for controlling yield of seed plant<sup>-1</sup>.



**Figure 3:** Biplot based on line x tester data in Brassica napus for seed yield explaining relationship among lines and testers

## Conclusion

Analysis of variance revealed significant variability among parental genotypes and their hybrid combinations for seed yield plant<sup>-1</sup>. Combining ability analysis based on biplot showed that among the female parents (lines) L-7 and L-6 whereas among the testers (T-1 and T-3) were found best and can be used in future breeding programs to produce high yielding hybrid as well as for cultivar development. Moreover, it is important to use the genotypes falling in different clusters in hybridization programs to produce cultivars with broader genetic base.

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### Author's Contribution

Ibni amin Khalil collected germplasm, developed breeding material; design field experiment collected analyzed data and prepared draft of the research article. Raziuddin supervised the entire research activities reviewed research article.

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