Research Article

# Evaluation of Genetic Diversity in Maintainer and Restorer Inbreds of *Helianthus annus* L. using Multivariate Techniques

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**Abstract** | Genetic diversity in Maintainer (B-line) and Restore (R-Lines) is a corner stone for the development of sunflower hybrid varieties. Seventy-seven of each, maintainer and restorer lines were sown in field and data were analyzed by using multivariate analysis. The results revealed that the mean values of all traits except for days to 50% flowering of maintainer lines were higher than restorer lines. The principal component analysis for B-lines showed that first eigen value equals 2.77 and represented 39.57 % of the total variability while second eigenvalue equals 1.24 and showed 17.76% of the total variability. The first two factors showed 57.34% of the initial variability. The principal component analysis for R-lines showed that the first eigenvalue equals 3.73 and represented 53.21% of the total variability while second eigenvalue equals 1.34 and showed 19.08% of the total variability. The first two factors showed 72.30 % of the total variability. Maximum inter-cluster distances were noted between cluster 2 and cluster 5 (51.221) for B-lines, and between cluster 3 and cluster 7 for R-lines (89.57). Considering inter-cluster distance within/between the lines and other agronomic traits, A/B and R-lines can be crossed to get maximum heterosis during hybrid development program.

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## Introduction

Genetic diversity and inter-relationship of breeding lines is not only valuable for germplasm conservation but also for the identification of inbred lines and selection of parental lines for hybrid production in sunflower (Darvishzadeh et al., 2010). Evaluation of germplasm and genetic diversity among species plays a significant role to speed up the breeding program of sunflower (Hallauer et al., 1988; Qamar et al., 2020). The importance of germplasm for the improvement of any crop was described for the first time by Nikolai Vavilov, who said that "The practical plant-breeder uses this material as bricks with which he must construct new forms".

Sunflower cultivation has complex and evolutionary history. However, the impact of these evolutionary events on genetic diversity is not fully understood (Mandel et al., 2011). Genetic diversity of sunflower germplasm is becoming narrow owing to commonly use of similar genetic resources (Zhang et al., 2005). The sunflower improvement can be achieved by using the hybrid vigour available in diverse parental lines.

The sunflower hybrids could give more yield and more resistant than open pollinated varieties.

The sunflower improvement can be achieved by development of hybrids depending on the phenomenon of hybrid vigour or heterosis available in diverse parental lines (Tyagi et al., 2018). Sunflower genepool has two different breeding types. R-line has homozygosity for dominant restorer alleles within the nucleus at one or more than one fertility restorer loci while B-line has homozygosity for non-restorer alleles. A-line has male sterile system and is maintained by B-line which has same nuclear make up. A-line and R-line is crossed to produce fertile hybrid with maximum heterosis. Therefore, it is necessary to maintain R-line and A/B lines as two different breeding pools. Evaluation and selection of inbred lines from different genetic resources used for the hybrid development is needed. Estimation of genetic diversity and determination of relationships among the collected germplasm from different sources increases the collection and its management (Nisar et al., 2008). The genetic diversity analysis helps the breeder to understand the evolutionary relationships among the genotypes (Liu et al., 2003). Multivariate statistical techniques are being widely used in morphological, biochemical and molecular marker based genetic analysis. Among these, principle components analysis (PCA), cluster analysis and principal coordinates(PCoA) are the most common and useful techniques (Kholghi et al., 2011). The objective of the study was to evaluate the genetic diversity of B-lines and R-lines (two types of inbreds) to get maximum heterosis during hybrid development programme.

## Materials and Methods

Seventy seven of each, maintainer and restorer lines were selected from sunflower gene pool maintained at Oilseeds Research Institute, Faisalabad (31˚26′S, 73˚06′E)), sown in field on 23th January and harvested on 15th May 2018 under semiarid climatic conditions. The experiment was arranged in Randomized Complete Block Design with three replications. Inter-plants and inter-rows distances were respectively 23 cm and 75cm. Recommended practices were adopted to have good plant stand and data were collected for days to 50% flowering, number of leaves, head diameter, plant height, stem thickness, days to maturity and yield per plant. Basic or descriptive statistics such as measure of central tendency and measure of dispersion for the above traits were calculated. Principal component analysis (PCA) and cluster analysis were used to evaluate/assess the pattern of variation in these traits using Xlstat statistical software.

## Results and Discussion

Sunflower inbred diversity comparison was made by using descriptive statistics (Table 1). The R-lines height was 2.35 cm (2.19%) greater on average than B-lines height. The restorer having average of 107.32 cm while B-lines having an average of 104.32cm. The standard deviation of R-lines data was 17.50 while B-lines data had 15.86. B-line had number of leaves were 3.74 (23.47%), greater on average than number of leaves in R-lines. The maintainer having an average of 19.67 for number of leaves and restore having an average of 15.93. The standard deviation of B-lines data was 3.76 while R-lines data 3.27.

**Table 1:** *Descriptive statistics for B-line and R-lines inbred lines.*

| Character | Inbred lines | Mean | SD | Variance | SE | Minimum | Maximum |
|---|---|---|---|---|---|---|---|
| Plant height(cm) | B-line | 104.97 | 15.86 | 251.57 | 1.82 | 66.60 | 137.40 |
| | R-line | 107.32 | 17.50 | 306.14 | 2.01 | 73.88 | 165.00 |
| Number of leaves | B-line | 19.67 | 3.76 | 14.10 | 0.43 | 11.80 | 34.00 |
| | R-line | 15.93 | 3.27 | 10.69 | 0.38 | 9.33 | 26.00 |
| Stem thickness | B-line | 20.84 | 2.95 | 8.67 | 0.34 | 14.41 | 34.23 |
| | R-line | 19.08 | 2.58 | 6.68 | 0.30 | 14.20 | 25.36 |
| Head size (cm) | B-line | 14.47 | 2.07 | 4.30 | 0.24 | 9.20 | 18.70 |
| | R-line | 8.27 | 3.62 | 13.09 | 0.41 | 4.33 | 20.00 |
| Days to flowering | B-line | 66.46 | 4.06 | 16.49 | 0.47 | 58.00 | 75.00 |
| | R-line | 68.09 | 3.11 | 9.66 | 0.36 | 59.00 | 78.00 |
| Days to maturity | B-line | 97.36 | 3.29 | 10.82 | 0.38 | 89.00 | 106.00 |
| | R-line | 95.17 | 6.48 | 42.01 | 0.74 | 81.00 | 111.00 |
| Yield/ Plant | B-line | 15.40 | 6.26 | 39.20 | 0.72 | 2.42 | 33.51 |
| | R-line | 7.63 | 5.39 | 29.09 | 0.62 | 0.65 | 28.10 |

The B-line data have more standard deviation than R-line data for the trait stem thickness (14.34%), days to flowering (30.54%) and yield (16.14%). While for the other trait head size (42.82%) and days to maturity (49.22%) the R-lines have more standard deviation than B-line.

The principal component analysis showed that the first two principal components had eigenvalue greater

than 1 for B and R lines. The scree plot of B-line and R-line showed that eigenvalue started after first and 2nd principle component respectively to form a straight line. These two principle component explained 57% and 72% from total variation for B-line and R-line respectively (Table 2). The cumulative proportion of these two components was 39% and 57% in B-line while 53% and 72% in R-line. In B-line loading plot, plant height, stem thickness, number of leaves, head size and yield per plant had large positive loading on component 1. In R-line loading plot, days to maturity, number of leaves, stem thickness, yield per plant, head size and days to 50% flowering also had large positive loading score on component 1 (Figure 1).

All the 77 B-lines and R-lines were grouped into seven clusters (Table 4). Among these seven clusters, cluster VI was the largest cluster having 19 genotypes while III was the smallest one containing only 2 genotypes. Similarly, for R lines, cluster II was the largest cluster, comprised of 37 genotypes while cluster VII was the smallest one which consisted only 1 genotype. The distance between the cluster centroid of B-line and R-line was indicated in Table 5. The divergent genotypes in B-lines and R-lines for days to 50% flowering and seed yield were grouped in cluster 3 and cluster 7, while cluster 5 and cluster 6 showed genetic variability for plant height. The head size differentiated between cluster 4 and cluster 1. The genotypes of cluster 7 gave maximum yield and genotypes of cluster 4 for head size can be crossed to get maximum heterosis and genetic variability. The genotypes of B-lines from cluster 3 and R-line from cluster 4 can be crossed to get early maturing hybrid. The genotypes from cluster 7 can also be crossed to get late maturing hybrids. Results indicated that for B-line the highest inter-cluster distances between cluster 2 and cluster 5 (51.22) followed by cluster 4 and cluster 5 (51.21), while minimum inter cluster distance was found between cluster 1 and cluster 7 (9.354). Maximum inter-cluster distances for R-lines was noted between cluster 3 and cluster 7 (89.57), followed by cluster 4 and cluster 7 (69.55).

**Table 2:** *Principal component analysis for various traits of sunflower inbred lines.*

| Character | B-lines | | R-lines | |
|---|---|---|---|---|
| | PC1 | PC2 | PC1 | PC2 |
| Plant height(cm) | 0.798201 | 0.04726 | 0.781129 | 0.40461 |
| Number of leaves | 0.745147 | -0.23689 | 0.815727 | 0.121735 |
| Stem thickness (cm) | 0.570338 | -0.06057 | 0.798519 | -0.11288 |
| Head size (cm) | 0.689007 | -0.29444 | 0.738332 | -0.56046 |
| Days to 50% flowering | 0.639694 | 0.594436 | 0.414333 | 0.815864 |
| Days to maturity | 0.417533 | 0.620531 | 0.819411 | 0.017793 |
| Yield/ Plant (g) | 0.440939 | -0.59676 | 0.65101 | -0.40557 |
| Eigenvalue | 2.770354 | 1.243254 | 3.72526 | 1.335826 |
| Variability (%) | 39.57649 | 17.76077 | 53.218 | 19.08322 |
| Cumulative % | 39.57649 | 57.33725 | 53.218 | 72.30122 |

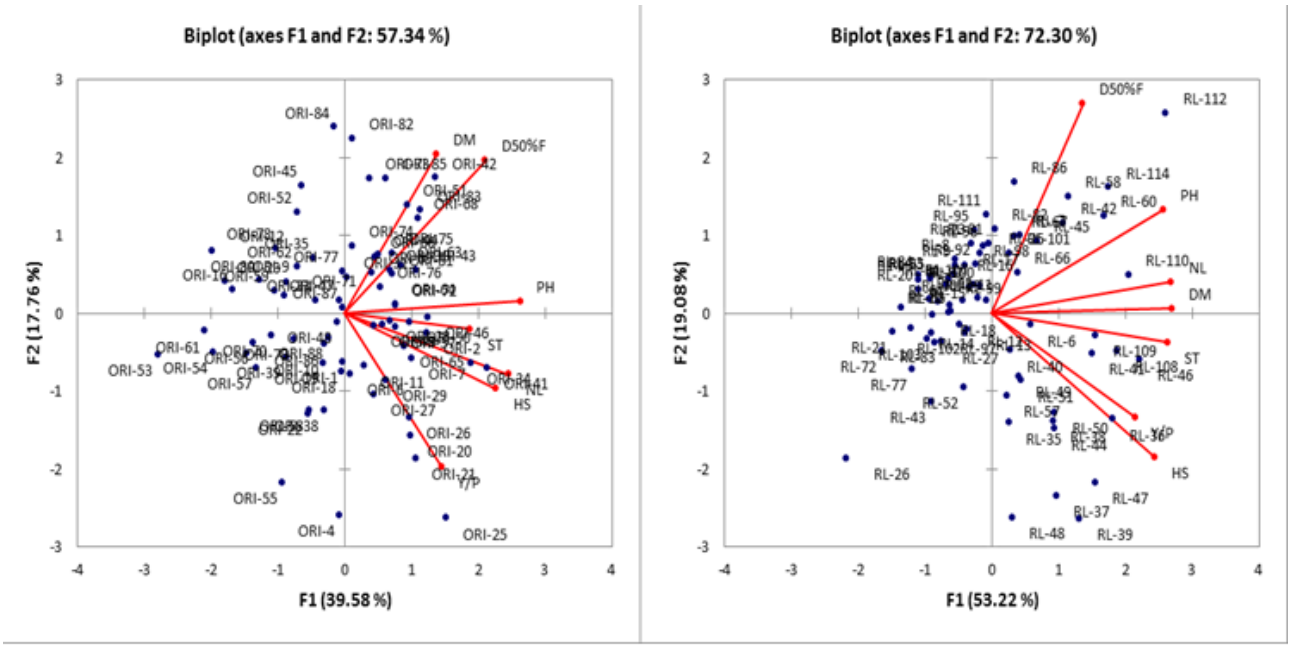


**Figure 1:** *Biplot of different morphological traits for B-lines and R-lines of sunflower.*

**Table 3:** *Cluster distribution of B-lines and R lines in sunflower (Helianthus annuus L).*

| S. No | Number of clusters | Number of B-lines | Number of R-lines | Name of B-lines | Name of R-lines |
|---|---|---|---|---|---|
| 1 | 1 | 13 | 11 | ORI-1, ORI-18, ORI-31, ORI-33, ORI-35, ORI-39, ORI-49 ORI-56, ORI-57, ORI-59, ORI-69, ORI-77, ORI-81 | RL-6, RL-7, RL-29, RL-45, RL-66, RL-67, RL-81, RL-82, RL-86, RL-98, RL-10 |
| 2 | II | 6 | 37 | ORI-2, ORI-7, ORI-20, ORI-25, ORI-20, ORI-25 | RL-8, RL-9, RL-11, RL-12, RL-12, RL-14, RL-16, RL-17, RL-18, RL-20, RL-23, RL-25, RL-27, RL-49, RL-52, RL-5, RL-61, RL-62, RL-64, RL-68, RL-69, RL-70, RL-77, RL-83, RL-84, RL-92, RL-93, RL-95, RL-96, RL-97, RL-99, RL-100, RL-102, RL-103, RL-107, RL-111 |
| 3 | III | 2 | 7 | ORI-4, ORI-22 | RL-10,RL-13,RL-15,RL-21, RL-26,RL-43,RL-72, |
| 4 | IV | 18 | 7 | ORI-6, ORI-8,ORI-29,ORI-30, ORI-34,ORI-37,ORI-41,ORI-42, ORI-44,ORI-46,ORI-50,ORI-5, ORI-65,ORI-72,ORI-73,ORI-68,ORI-75 | RL-35,,RL-37,RL-38,RL-39,RL-48,RL-51,RL-57 |
| 5 | V | 5 | 10 | ORI-9,ORI-12,ORI-23, ORI-53,ORI-54 | RL-36,RL-41,RL-42, RL-44,RL-47,RL-50, RL-58,RL-60,RL-108,RL-109 |
| 6 | VI | 19 | 3 | ORI-10,ORI-16,ORI-47, ORI-48, ORI-62, ORI-58,ORI-55,ORI-52, ORI-88,ORI-87, ORI-84,ORI-82, ORI-80,ORI-79,ORI-78,ORI-71,ORI-70 | RL-46,RL-110,RL-114 |
| 7 | VII | 13 | 1 | ORI-10,ORI-16,ORI-47, ORI-48, ORI-88,ORI-87,ORI-84,ORI-82, ORI-80,ORI-79, ORI-78,ORI-71,ORI-70 | RL-112 |

**Table 4:** *Inter-cluster distance between cluster centroids in B-lines (upper diagonal) and R-lines (lower diagonal).*

| No. of clusters | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 | Cluster 5 | Cluster 6 | Cluster 7 |
|---|---|---|---|---|---|---|---|
| Cluster 1 | 0 | 21.3308 | 29.1274 | 18.6178 | 32.9878 | 14.579 | 9.3538 |
| Cluster 2 | 23.758 | 0 | 39.419 | 11.7538 | 51.2207 | 32.4036 | 16.0577 |
| Cluster 3 | 45.204 | 21.657 | 0 | 44.1303 | 21.6458 | 18.8342 | 26.6196 |
| Cluster 4 | 28.624 | 14.219 | 24.806 | 0 | 51.2136 | 32.2324 | 17.9627 |
| Cluster 5 | 13.813 | 26.348 | 46.232 | 24.043 | 0 | 19.3309 | 35.7145 |
| Cluster 6 | 25.894 | 48.164 | 69.475 | 48.833 | 25.440 | 0 | 16.7399 |
| Cluster 7 | 45.216 | 67.998 | 89.477 | 69.552 | 45.875 | 21.472 | 0.000 |

**Table 5:** *Cluster means for different variables of B-lines and R-lines in sunflower genotypes.*

| Clusters | Cluster 1 | | Cluster 2 | | Cluster 3 | | Cluster 4 | | Cluster 5 | | Cluster 6 | | Cluster 7 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variables | B-line | R-line | B-line | R-line | B-line | R-line | B-line | R-line | B-line | R-line | B-line | R-line | B-line | R-line |
| Plant height(cm) | 105.95 | 124.17 | 120.53 | 100.94 | 81.80 | 79.95 | 123.27 | 97.86 | 73.64 | 120.91 | 91.82 | 145.58 | 106.29 | 165.00 |
| Number of leaves | 19.49 | 16.97 | 23.87 | 14.61 | 22.10 | 12.52 | 21.99 | 16.67 | 15.32 | 18.83 | 16.89 | 22.89 | 20.03 | 22.33 |
| Stem thickness(cm) | 20.53 | 19.27 | 22.24 | 17.73 | 20.92 | 17.22 | 21.30 | 21.68 | 17.17 | 21.69 | 20.40 | 22.88 | 21.92 | 24.41 |
| Days to 50% flowering | 64.31 | 69.55 | 67.67 | 67.78 | 61.00 | 66.14 | 69.22 | 64.71 | 62.80 | 69.70 | 65.95 | 70.33 | 67.23 | 78.00 |
| Head size(cm) | 14.18 | 6.49 | 15.39 | 6.41 | 14.50 | 6.57 | 15.74 | 13.14 | 12.46 | 13.07 | 13.21 | 13.11 | 15.18 | 12.00 |
| Days to maturity | 96.69 | 94.18 | 97.33 | 92.46 | 96.00 | 87.86 | 98.28 | 99.86 | 94.80 | 103.80 | 96.53 | 105.00 | 99.15 | 109.00 |
| Yield/plant (g) | 11.43 | 8.32 | 25.82 | 5.00 | 27.13 | 4.70 | 14.74 | 12.97 | 8.88 | 14.24 | 12.97 | 9.86 | 19.76 | 7.59 |

For the maintenance, evaluation and utilization of germplasm successfully, it is necessary to know the range of existing genetic diversity (Mohammadi and Prasanna, 2003). Lee et al. (2004) studied the morphological traits and indicated that characterization is an important step in the classification of germplasm because breeding program depends on the extent of genetic variation (Tantasawat et al., 2010).

Simple statistics such as mean and standard deviation had also been used for genetic diversity analysis in some crops like ground nut (*Arachis hypogaea* L.) (Ntundu et al., 2006), garlic (*Allium sativum* L.) (Panthee et al., 2006) and melon (*Cucumis melo* L) (Lotti et al., 2008). Similar results were also reported in sunflower by (Sujatha and Nandini, 2002; Yasumoto et al., 2012; Avin et al., 2013).

Measure of central tendency showed that R-lines height data was more spread out or dispersed than B-lines data. The height of B-lines were more consistent (closer to the mean). The R-lines had large variance and B-lines had a small one which indicated that data in R-lines were far from the mean and from each other, indicating larger diversity within R lines samples. However, the standard deviation for number of leaves indicated that B-lines were more dispersed than R-lines. Similarly stem thickness, days to 50% flowering and yield were more spread out over wide range in B-line.

Principal component analysis was used to know the most significant variable in data set. From the scree plot of both type inbreds it was clear that eigenvalue started after first and 2$^{nd}$ principle component to form a straight line, therefore these two principal components explained the highest percentage/part variation. We conclude that these components have major proportion for genetic variation than other two components. The genetic diversity in sunflower hybrids and inbred lines (B-line and R-line) using principal component analysis has been studied by earlier researchers (Zeinalzadeh-Tabrizi et al., 2018; Hongtrakul et al., 1997; Darvishzadeh et al., 2010). In B-line loading plot, component 1 measures long term stability in yield production.

Cluster analysis has also been used to divide the genotypes and environments into homogeneous categories (Piepho, 1998). Cluster distribution can categorize a large number of germplasm into different homogenous group (Razzaque et al., 2016). The seven cluster with different number of genotypes indicated that there is good extent of genetic diversity in both group B-lines and R-lines. The genotypes within the group presented less range of genetic variability, while different clusters indicated wider range of variability. It is prerequisite to find the genotypes with more than one desirable traits from different groups based on the mean values of their cluster. It has been indicated by (Binodh et al., 2006; Anandakumar et al., 2010; Chandirakala and Manivannan, 2014) that involving the divergent parents from divergent clusters is important to get the superior heterosis.

## Conclusions and Recommendations

The genetic diversity was observed in B-lines and R-lines for agronomic traits. The inbred lines having distance among the clusters of two types inbreds is more reliable than inter cluster distance within one type inbred. Therefore, using more diverse inbreds maximum heterosis can be produced during the hybrid development program.

## Novelty Statement

The descriptive statistics along with the multivariate analysis are more reliable to study the genetic diversity of two types sunflower inbred lines.

## Author's Contribution

**Hafiz Basheer Ahmad:** Research and wrote manuscript.
**Sajida habib, Rizwana Qamar and Salsabeel Rauf:** Contributed in Research.
**Waqas Shafaqat Chattha:** Analyzed the data.
**Shazia Saeed:** Recorded the data.
**Abdul Khaliq:** Edited and reviewed the manuscript.

*Conflict of interest*
The authors have declared no conflict of interest.

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