

Original Research Article

Analysis of morphological variation, grouping and path coefficient studies in a set of maize inbred lines local to North East Hill Region of India

Abstract:

In the current study a total of 191 maize inbred lines were set up in an augmented randomized complete block design to study the genetic variation and interrelationship of different yield contributing parameters. Observations were recorded on a total of nine phenological and ear / kernel related traits. Significant results for ANOVA indicated presence of substantial variation while the first three axes of PCA could explain 75.54% of the variation present. Ear and flowering traits were orthogonal to each other and the highest variation in PC1 could be attributed to total grain weight. This was also reflected in the Agglomerative Hierarchical Clustering which grouped the inbreds into five clusters primarily on the basis of total grain weight which accounted for the highest variation between the clusters. Substantial heritability genetic advance as percentage of mean were observed for total grain weight indicating that selection for grain weight would be fruitful. Association and path coefficient studies after adjustments for multi-collinearity using k constant method (0.05) revealed that total grain weight was highly and positively correlated with ear weight, number of grain kernel rows and seed index. Therefore, simultaneous selection for these traits would be useful. Overall, our studies go on to show presence of sufficient genetic variability in the inbreds under study and hybridization between inbred lines grouped in different clusters have a high chance of producing heterotic hybrids.

Key words: *Maize, inbreds, Genetic variation, Correlation, Path analysis*

1 Introduction:

Successful crop improvement programmes depend on effective utilization of genetic diversity found in crop plants. Crop diversity in turn is the outcome of interventions both by the environment and local farmers [1]. Among cereals, maize possesses a high degree of genetic diversity having undergone extensive modification both since domestication and following migration to different parts of the world [2]. Today, maize is a multipurpose crop with cultivation spanning from approximately 50°N to 45°S latitudinally and broadly classified as temperate, subtropical and tropical germplasm [3, 4].

Hybrid maize development first began in the early 1900s in the USA with landmark contributions from maize breeders who successfully explored the concept of inbred-hybrid development in maize utilizing local germplasm. First proposed by Shull [5] while discussing the composition of open pollinated field maize, this concept focused on inbred development following successive rounds of selfing. Inbred lines in maize can be derived from a multitude of source populations including landraces and have been a rich resource for both fundamental and applied investigations [6].

A general consequence of inbreeding in maize is that it leads to a high degree of uniformity within the lines and high variability between the lines even for inbreds originating from the same parental stock. This variability is the result of fixation of allelic combinations at different loci [7]. Inbreds are highly influenced by environment and identification of stable inbreds is crucial for hybrid development programmes. Such programmes require that a large number of segregating families be critically evaluated in order to identify those with greater yield potential. Evaluation on the basis of cobs with higher grain weight is practical since it ensures availability of seeds at later stages of breeding programmes given that successive selfings lead to inbreeding depression in maize. However, the outcome of grain weight could be due to the direct/indirect influence of other yield contributing traits. Therefore, besides an understanding of the type of variation present, knowledge of the interrelationship between traits which influence the genetic potential of inbreds is also required. Use of path analysis first proposed by Wright [8] is one such scientific method of analysis which allows the elucidation of the direct and indirect effects influencing the association shared among heritable components [9, 10].

Keeping in mind the necessity to understand factors governing variation and heritability, the current study was framed to evaluate a set of inbreds developed from germplasm local to North East India. An attempt to understand the factors contributing to variation in the experimental material using Analysis of Variance (ANOVA), Principal Component Analysis (PCA) and heritability was followed by path analysis studies. Since the inbreds developed were a collection of different parental stock, genetic diversity analysis using agglomerative hierarchical clustering method was also taken up.

2 Materials and Methods:

2.1 Plant material:

The current study included a total of 191 maize inbred lines. These inbreds had been developed following three rounds of sibbing followed by two rounds of selfing from a selection of landraces local to North Eastern Hill Region of India -an important secondary center of maize diversity in Asia [11].

2.2 Field layout:

The experiment was laid out in an augmented randomized complete block design consisting of two blocks (Figure 1). Three commercial checks were repeated thrice at random within each block. The inbreds were sown in single rows with a row and plant to plant spacing of 60 x 30cm and the data on three plants within each row was considered for analysis. All the recommended intercultural practices were taken up and need based application of fertilizers and pesticides was done.

| Block-1 | | | | | Block-2 | | | | |
|------------|---------|---------|---------|---------|---------|---------|---------|---------|------------|
| Border row | | | | | | | | | |
| Check-3 | | | | | | Check-1 | | | Check-3 |
| Inbred-1 | | | | | | | | | |
| Inbred-2 | | Check-1 | | Check-2 | | | | | |
| Inbred-3 | | | | | | | | | |
| Inbred-4 | | | | | | | Check-1 | | |
| Inbred-5 | | | | | | | | | |
| | | | Check-3 | | | | | | Check-3 |
| | | | | | | | | | |
| | Check-2 | | | | Check-3 | Check-2 | | | |
| | | | | | | | | | |
| | | | | | | | Check-3 | | |
| | | | | | | | | | Inbred-187 |
| | | Check-1 | | | | | | Check-2 | Inbred-188 |
| | | | | | | Check-1 | | | Inbred-189 |
| | | | | | | | | | Inbred-190 |
| | | | | | | | | | Inbred-191 |
| Check-2 | | | | | | | | | Check-2 |
| | | | | | | | | | Border row |
| | | | | Check-1 | | | | | |
| Border row | | | | | | | | | |

Figure 1. Experimental plot layout of 191 inbred lines and three checks studied using an augmented block design

2.3 Observations recorded:

Observations on days to 50 percent tasseling (DT) and days to 50 percent silking (DS) were recorded for calculating the anthesis silking interval (ASI). Ear related traits -cob length (CL) (cm), cob diameter (CD) (cm), ear weight (EW) (g), number of kernel rows per cob (NGKR), 100-seed weight (SI) (g) and grain yield per plant (TGW) (g) were recorded for further statistical analysis.

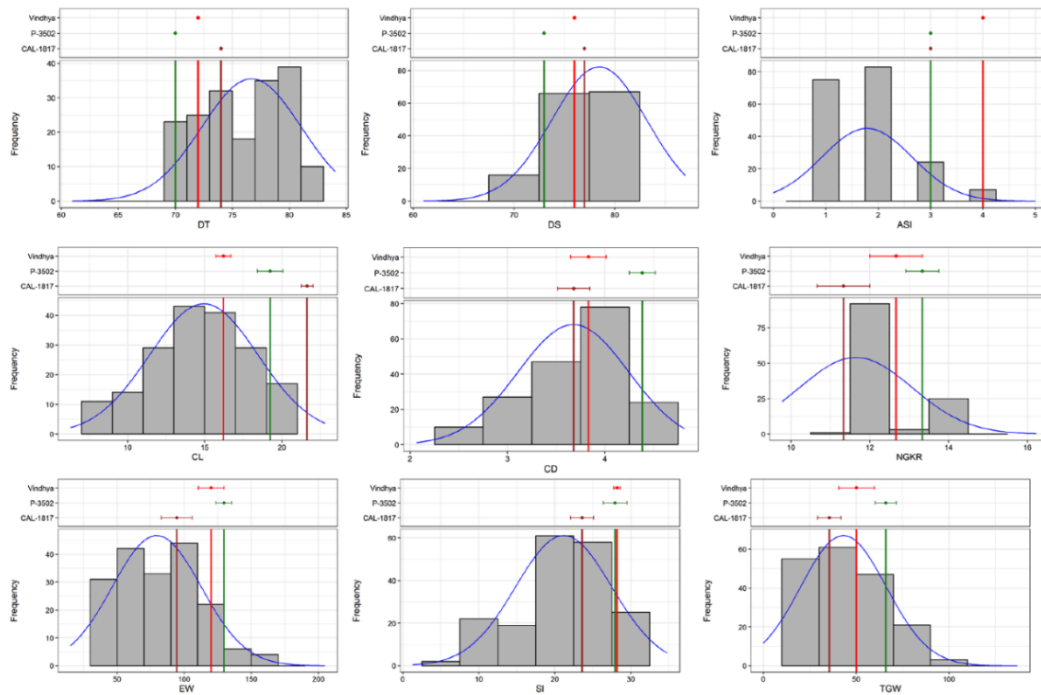
2.4 Statistical analysis:

Mean and variance were calculated. The analysis of variance (ANOVA), heritability, and genetic advance as percentage of mean were calculated using “augmentedRCBD.bulk” function of ‘augmentedRCBD’ package [12] in R software [13]. Principal Component analysis was done using XLSTAT software (Version 2016.02.28451). Agglomerative clustering of **ward’s method** was done by scaling the observation variables (mean of zero and standard deviation of 1) to avoid any bias. Function ‘hclust’ of R software using “clv” package [14] of R software was used to calculate cluster distances. The Pearson correlation matrix was computed using the “corr_coef” function and the correlation plot constructed using the “corr_plot” function of ‘metan’ package [15] in R software [13]. The correlation matrix generated was analysed for multicollinearity following which path analysis studies under collinearity (k constant method) was taken up using the GENES software [16].

3 Results and Discussion:

A study of the mean performance and distribution (Figure 2) revealed that traits total grain weight, ear weight and number of grain kernel rows were significantly and positively skewed with higher number of individuals concentrated around the lower values while mean values for days to flowering were significantly skewed towards the larger values. This is

expected since inbreeding depression in a highly allogamous species like maize is known to reduce the reproductive fitness [7, 17, 18] and it would practically take more than one generation of open pollination to restore these lines to their original levels of heterozygosity [7, 19]. Exceptions were observed for cob diameter and seed index which despite being associated with reproductive fitness, were negatively skewed while values for cob length appeared to follow a normal distribution. Highest variance was observed for ear and total grain weight and seed index - all three being major yield contributing traits. The lowest variation was detected for number of grain kernel rows. ANOVA studies revealed significant variation at 0.05 significance level of significance for seven quantitative traits with flowering days, anthesis silking interval, cob length and seed index also significant at 0.01 significance level (Table 1). Significant F-values implied presence of variability between the lines under study. Further analysis using PCA revealed that the first three axes accounted for 75.54% of the total variation in the data. All ear related traits barring cob length loaded heavily on PC1. As revealed by squared cosine values of the variables, total grain weight accounted for the highest percent contribution in this axis. Flowering traits were orthogonal to the ear related traits and loaded heavily on PC2. The highest loading for cob length was observed for PC3 (Table 2).



DT: Days to 50 % tasseling; DS: Days to 50 % silking; ASI: Anthesis Silking Interval; CL: Cob length; CD: Cob diameter; EW: Ear weight; NGKR: Number of kernel rows per cob; SI: 100-seed weight; TGW: Total grain weight

Figure 2 Mean performance and variance of the nine yield contributing traits studied for the 191 inbred lines along with checks varieties Vindhya (red), P-3502 (green) and CAL-1817 (burgundy). The y-axis represents the frequency of the individuals for each of the parameters studied

Table 1 Mean square values for the nine yield contributing traits under study

| Source of Variation | df | DT | DS | ASI | EW | CD | CL | NGKR | SI | TGW |
|---------------------|----|----|----|-----|----|----|----|------|----|-----|
|---------------------|----|----|----|-----|----|----|----|------|----|-----|

| | | | | | | | | | | |
|---------------------------------------|------------|-----------|-----------|------------|-----------|-----------|-------------|-------|----------|------------|
| Block (ignoring Treatments) | 1 | 316 ** | 293 ** | 0.23 ** | 2663 * | 0.1465 | 2.53 | 9.2 | 0.42 | 2467 ** |
| Treatment (eliminating Blocks) | 193 | 19 ** | 21 ** | 0.92 ** | 1201 * | 0.3353 | 13.28 ** | 2.145 | 41 ** | 518 * |
| Treatment: Check | 2 | 24 ** | 26 ** | 2 ** | 1972 * | 0.82 * | 44.47 ** | 6.222 | 40 * | 1388 * |
| Treatment: Test and Test vs. Check | 191 | 19 ** | 21 ** | 0.92 ** | 1193 * | 0.3302 | 12.96 ** | 2.102 | 41 ** | 509.6 |
| Residuals | 14 | 0 | 0 | 0 | 525.7 | 0.1657 | 1.66 | 2.222 | 9.77 | 248.9 |

* $P = \leq 0.05$; ** $P = \leq 0.01$

DT: Days to 50 % tasseling; DS: Days to 50 % silking; ASI: Anthesis Silking Interval; CL: Cob length; CD: Cob diameter; EW: Ear weight; NGKR: Number of kernel rows per cob; SI: 100-seed weight; TGW: Total grain weight

Table 2 Principal Component Analysis for the nine yield contributing traits under study

| Factor loadings: | | | | Contribution of the variables (%): | | | Squared cosines of the variables: | | |
|------------------|-------------|-------------|-------------|------------------------------------|--------------|--------------|-----------------------------------|-------------|-------------|
| Traits | PC1 | PC2 | PC3 | PC1 | PC2 | PC3 | PC1 | PC2 | PC3 |
| DT | 0.04 | 0.94 | 0.00 | 0.04 | 37.55 | 0.00 | 0.00 | 0.88 | 0.00 |
| DS | 0.02 | 0.98 | 0.04 | 0.01 | 40.98 | 0.21 | 0.00 | 0.96 | 0.00 |
| ASI | -0.11 | 0.65 | 0.24 | 0.36 | 18.29 | 6.70 | 0.01 | 0.43 | 0.06 |
| EW | 0.88 | -0.03 | 0.09 | 22.25 | 0.04 | 0.94 | 0.78 | 0.00 | 0.01 |
| CD | 0.71 | 0.15 | -0.03 | 14.42 | 1.02 | 0.10 | 0.51 | 0.02 | 0.00 |
| CL | 0.47 | -0.21 | 0.82 | 6.40 | 1.90 | 78.94 | 0.23 | 0.04 | 0.67 |
| NGKR | 0.71 | 0.05 | -0.25 | 14.38 | 0.13 | 7.55 | 0.51 | 0.00 | 0.06 |
| SI | 0.82 | -0.04 | -0.07 | 19.10 | 0.05 | 0.52 | 0.67 | 0.00 | 0.00 |
| TGW | 0.90 | 0.03 | -0.21 | 23.03 | 0.04 | 5.05 | 0.81 | 0.00 | 0.04 |

DT: Days to 50 % tasseling; DS: Days to 50 % silking; ASI: Anthesis Silking Interval; CL: Cob length; CD: Cob diameter; EW: Ear weight; NGKR: Number of kernel rows per cob; SI: 100-seed weight; TGW: Total grain weight

Agglomerative Hierarchical clustering using Ward's method divided the 191 inbred lines into five clusters (Figure 3). The largest inter cluster distance was recorded for clusters I and III (5.63) with these clusters having grouped inbreds with highest and lowest respective

values for the ear related traits. The lowest inter-cluster distance was observed between cluster IV and V (3.57) (Table 3). The highest contribution to variation in PC1 came from individuals grouped in Cluster I with most of the lines having recorded ear and total grain weight on the higher side. The genetic advance calculated as percentage of mean ranged from medium in case of cob diameter (15%) to high (40-55%) for the remaining ear related traits (Figure 4). A high genetic advance implied that selection for these traits would have a genetic basis despite being influenced by environment. With respect to number of grain kernel rows, the variance was highly non-significant and therefore genetic advance could not be calculated for this trait.

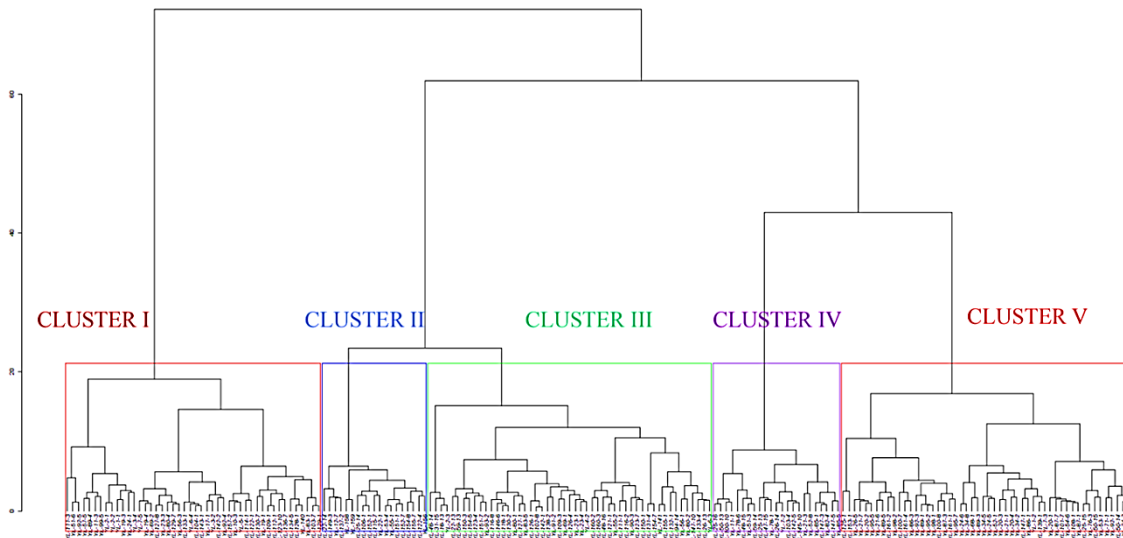
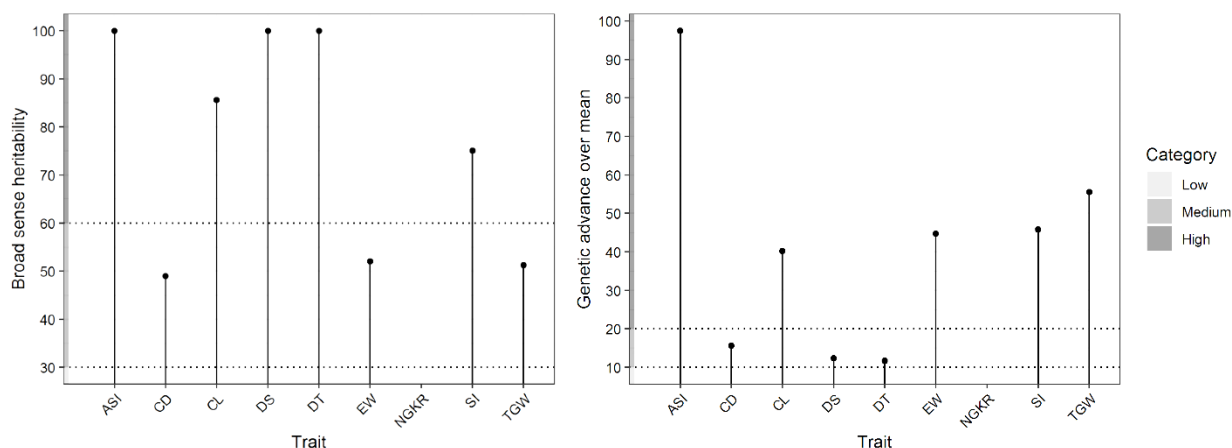


Figure 3 Clusters based on Agglomerative Hierarchical Clustering with Euclidean Distances using Ward's method. A total of five cluster were generated

Table 3 Inter and intra cluster distances of the five clusters generated based on the nine quantitative yield traits studied

| | No. of inbreds | Cluster-1 | Cluster-2 | Cluster-3 | Cluster-4 | Cluster-5 |
|-----------|----------------|-----------|-----------|-----------|-----------|-----------|
| Cluster-1 | 46 | 3.11 | 4.07 | 5.63 | 4.56 | 3.79 |
| Cluster-2 | 52 | 4.07 | 3.23 | 4.18 | 4.25 | 4.30 |
| Cluster-3 | 23 | 5.63 | 4.18 | 2.61 | 3.83 | 4.78 |
| Cluster-4 | 51 | 4.56 | 4.25 | 3.83 | 3.06 | 3.57 |
| Cluster-5 | 19 | 3.79 | 4.30 | 4.78 | 3.57 | 2.58 |



ASI: Anthesis Silking Interval; CD: Cob diameter; CL: Cob length; DS: Days to 50 % silking; DT: Days to 50 % tasseling; EW: Ear weight; NGKR: Number of grain kernel rows; SI: 100-seed weight; TGW: Total grain weight

Figure 4 Broad sense heritability (hBS) and genetic advance as percentage of mean (GAM) and for each of the nine parameters studied

Besides knowledge of variation and heritability, an understanding of the degree of correlation among traits is important for formulating selection strategies in crop improvement. The Pearson correlation, regarded as the strength of the linear association between two traits [20, 21] is one of the most commonly used tools for estimating such associations [22-24]. Pearson's correlation studies revealed that total grain weight with maximum variability in PC1 and a high genetic advance was highly and positively correlated with cob diameter (0.55), ear weight (0.78), number of grain kernel rows (0.63) and seed index (0.74). Maize grain yield is the outcome of ear length, ear diameter and thousand-kernel weight which are known to be significantly correlated with each other and influence the final outcome [25].

Use of path coefficients- a form of standardized partial regression analysis can further supplement the information gained from correlation studies by splitting correlation values into direct and indirect effects. Conventional path analysis for the present study however indicated presence of multicollinearity in the estimation of path coefficients. The direct effects of days to tasseling (-0.30) and that to silking (0.35) were high in magnitude but opposite in direction. Multi-collinearity generates bias in the estimation of path coefficients, especially in traits with large VIF (Variance Inflation Factors) (Table 4). Very high variance inflation factors (VIF) were observed for traits DT, DS, and the ASI, respectively and therefore collinearity studies using the k constant approach ($k = 0.05$) was taken up. Path coefficients after multicollinearity adjustments revealed that traits ear weight (0.40), seed index (0.32) and number of grain kernel rows (0.24) in that order exhibited the largest direct and positive effect on total grain weight. With respect to ear weight, besides a high direct effect, indirect effect via seed index and cob diameter was also observed to influence the outcome of total grain weight (Table 5).

Table 4 Estimates of direct effects on total grain weight and measures of multicollinearity diagnosis

| Trait | Conventional path analysis | | Path analysis under collinearity (K=0.05) | |
|-------|----------------------------|--------------|---|-------|
| | Direct effects | VIF | Direct effects | VIF |
| DT | -0.30 | 2483264605.5 | 0.013 | 10.38 |
| DS | 0.35 | 2933107990.6 | 0.008 | 12.02 |
| ASI | -0.08 | 93472538.4 | -0.023 | 1.64 |
| EW | 0.44 | 2.5 | 0.408 | 2.35 |
| CD | 0.07 | 1.6 | 0.084 | 1.58 |
| CL | -0.12 | 1.3 | -0.097 | 1.31 |
| NGKR | 0.25 | 1.4 | 0.248 | 1.46 |
| SI | 0.33 | 1.9 | 0.327 | 1.88 |

DT: Days to 50 % tasseling; DS: Days to 50 % silking; ASI: Anthesis Silking Interval; CL: Cob length; CD: Cob diameter; EW: Ear weight; NGKR: Number of kernel rows per cob; SI: 100-seed weight; TGW: Total grain weight

Table 5 Estimates of Pearson's correlation coefficients and of the direct and indirect path coefficients of the respective traits on total grain weight after adjustments for multicollinearity

| | Correlation (r) coefficients with TGW | Direct effect | Indirect effect via | | | | | | | |
|------|---------------------------------------|---------------|---------------------|--------|--------|--------|-------|--------|--------|--------|
| | | | DT | DS | ASI | EW | CD | CL | NGKR | SI |
| DT | 0.05 | 0.015 | 0 | 0.005 | -0.008 | 0.008 | 0.011 | 0.012 | 0.016 | -0.008 |
| DS | 0.03 | 0.005 | 0.015 | 0 | -0.011 | -0.001 | 0.010 | 0.013 | 0.012 | -0.010 |
| ASI | -0.08 | -0.022 | 0.006 | 0.003 | 0 | -0.047 | 0.002 | 0.010 | -0.016 | -0.018 |
| EW | 0.77** | 0.407 | 0 | 0 | 0.003 | 0 | 0.046 | -0.044 | 0.128 | 0.218 |
| CD | 0.55** | 0.084 | 0.002 | 0.001 | 0 | 0.223 | 0 | -0.023 | 0.101 | 0.161 |
| CL | 0.25** | -0.098 | -0.002 | -0.001 | 0.002 | 0.181 | 0.020 | 0 | 0.054 | 0.098 |
| NGKR | 0.63** | 0.248 | 0.001 | 0 | 0.001 | 0.209 | 0.034 | -0.021 | 0 | 0.146 |
| SI | 0.73** | 0.328 | 0 | 0 | 0.001 | 0.270 | 0.041 | -0.029 | 0.110 | 0 |

DT: Days to 50 % tasseling; DS: Days to 50 % silking; ASI: Anthesis Silking Interval; CL: Cob length; CD: Cob diameter; EW: Ear weight; NGKR: Number of kernel rows per cob; SI: 100-seed weight; TGW: Total grain weight

Figures in bold indicate the high direct effects by the respective explanatory variables on the dependent variable

Insights into the interrelationships between major yield parameters and their associated independent variables is necessary for effective selection especially in traits with low to medium heritability [26]. Path analysis under multicollinearity has been successfully used to understand associations between yield contributing traits after adjustments to address the negative impact of multicollinearity [27-30]. For the present study, ear weight, number of grain kernel rows and seed index appear to have a high direct effect on total grain yield. Therefore, simultaneous selection of these traits when selecting for higher grain weight would prove useful.

4 Conclusion:

Based on studies on variance components, and genetic parameters the present study revealed presence of substantial variability in the inbreds especially for ear related traits. Accordingly, cluster analysis grouped the inbreds with contrasting ear and total grain weight into the most distant clusters. Hybridization between these contrasting lines from different clusters may be expected to yield heterosis and also produce transgressive segregation during population development. Path analysis after adjustments for multicollinearity revealed that simultaneous selection for ear weight, grain kernel rows and seed index need to be considered to enhance the heritability of total grain weight.

DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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