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Assessment of Productivity Potential of Doubled Haploid Lines in Maize (*Zea mays* L.) for Suitability as Parents in Hybrid Development

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Abstract

The present study was performed to quantify the amount of variation present in 275 doubled haploid lines developed from the cross CV-29073758 × CV-29073679 and also to understand the distribution pattern for seven yield related traits in maize. Analysis of variance revealed mean squares attributable among and between the DHs and the checks were significant for all the traits. These results suggested significant differences among the maize doubled haploid lines. All the quantitative traits exhibited less than three kurtosis value indicating that the tails of the distribution of these traits are platykurtic. It indicated that genetic control of these traits was influenced by many genes, with the majority of them displaying complementary epistasis with increasing effects. Correlation studies revealed that kernel weight per cob had a significant positive correlation with cob length, cob girth, kernel rows per cob ($p<0.001$) followed by plant height ($p<0.01$) and ear height ($p<0.05$). It indicated that kernel weight per cob can be increased by giving importance to these traits while formulating selection criteria in yield improvement programmes. In the Principal Component Analysis, the first principal component (PC1) showed 30.85 per cent while, PC2 and PC3 exhibited 21.25 per cent and 15.10 per cent variation respectively, among the doubled haploid lines for the traits under study. As indicated by the principal component analysis, the K-mean clustering resulted in three clusters with the cluster B consisted of 118 DH lines followed by cluster A with 110 DH lines. The DH lines with highest values for plant height, ear height, cob girth, cob length, kernel weight per cob and test weight were formed in cluster C. The study was helpful in identifying several superior DH lines which can be used as parents in hybrid development programmes.

Keywords : Maize, Doubled haploids, Correlation, Complementary epistasis, Platykurtic distribution, Principal component analysis, K- means clustering

Maize is one of the most important cereal crops of the world and contributes to food security in most of the developing countries. In India, maize is the third most important crop after rice and wheat. Currently, nearly 1147.7 million MT of maize is being produced together by over 170 countries from an area of 193.7 million ha with average productivity of 5.75 t/ha. As a rapid generation advancement tool, doubled haploid (DH) has decreased the time needed to create maize inbreds from six to seven selfing generations to just two generations. The rapid development of DH lines helps in more reliable selection than lines obtained through consecutive self-pollination because in DH whole genome is duplicated and all its genetic loci are homozygous (Couto *et al.,* 2019).

Morphological characterization and grouping of germplasm is very important to study relationships among cultivars and to identify accessions with desirable traits and also it helps the breeders to avoid duplication in sampling populations. Among the most statistical tools, skewness and kurtosis are more powerful which help us to understand relative mean performance, nature of distribution of traits and for characterizing the nature of epistasis. Fasoulas (1981) considered that departure from normal distribution is an indication of the presence of unfavourable genes that a breeder has to remove during the selection procedure. Yield is a complex trait and is influenced by several component traits and we need to identify those characters that contribute to improvement of kernel yield. Correlation studies provide knowledge of association among different characters and kernel yield (Greveniotis *et al*., 2017).

The principal component analysis is used to evaluate the magnitude of genetic diversity among the germplasm (Guedira, 2000) and cluster analysis is used to classify maize (*Zea mays* L.) accessions and can be used by breeders and geneticists to identify subsets of accessions which have the potential utility for specific breeding or genetic purposes (Rincon *et al*., 1996). The present study was carried out to quantify the genetic variability in maize DH lines, to work out the contribution of different characters to divergence and to classify the DH lines employing K-means clustering.

MATERIAL AND METHODS

Basic Genetic Material and Development of Doubled Haploid Population

The basic genetic material consisted of 275 maize doubled haploid lines developed from the cross CV-29073758 × CV-29073679 along with parental inbreds. The susceptible inbred line (CV-29073758) was crossed with resistant line (CV-29073679) during *kharif* 2020 and F_1 was selfed to obtain F_2 plants. These F_2 plants were crossed with male haploid inducer inbred. The dominant grain purple colour marker gene (R1-nj marker) was employed to separate haploid kernels without pigmentation on embryo and those with pigmentation as regular diploids. The haploid kernels thus separated were placed in the paper towels for germination. When the coleoptiles were about 2-cm long the tip was cut-off and submerged in colchicine solution with Dimethyl Sulphoxide (DMSO). The seedlings were washed under tap water and planted in biodegradable ellepots filled with peat pellets. These pots were kept in the shade house till three-leaf stage and then transplanted in the DH nursery net house and selfed to obtain doubled haploids (DH_1). This process resulted in 275 doubled haploid lines from F_2 plants of the cross CV-29073758 × CV-29073679.

Field Layout

The 275 maize inbred lines and their respective parents as checks were evaluated in the augmented design and checks were repeated after every 20th row of test entries. All the entries were planted in rows spaced 0.60 m apart with an intra-row spacing of 0.20 m at the Mega Breeding Station, Bayer Crop Science Pvt. Ltd., Kallinayakanahalli, Gauribidanur (T), Chikkaballapur (D) 13.464013^0 N, 77.519178^0 E during, 2021 *kharif.* The observations were recorded on five plants in all 275 doubled haploid lines for plant height (PH), ear height (EH), cob girth (CG), cob length (CL), number of kernel rows per cob (KR), kernel weight per cob (KW) and test weight (TW).

Statistical Analysis

The mean of five plants was used to perform the analysis of variance as per augmented design. Descriptive statistics was employed to study the distribution pattern of DH lines with respect to quantitative traits and analysed using R software. Skewness was worked out by using Agostino test and kurtosis and that of fourth-degree statistics by using Anskombi test.

Three types of kurtosis recognised based on the values which depends on the distribution curve.

If kurtosis value = 3 = Normal curve = Mesokurtic

If kurtosis value > 3 = leaping curve = Leptokurtic

If kurtosis value < 3 = Flat curve = Platykurtic

Similarly, the lack of symmetry *i.e.,* skewness was recognised based on the coefficient of skewness values which ranged from -3 to +3. The type of distribution based on the skewness values are as follows.

If skewness value is zero = symmetrical distribution

If skewness value is negative = negatively skewed distribution

If skewness value is positive = positively skewed distribution

Correlation, principal component analysis and K means clustering were performed by using factoshiny and factoextra packages in R software. (Pauline Vaissie, 2021 not found in references).

RESULTS AND DISCUSSION

Analysis of variance revealed highly significant differences among doubled haploid lines for all the traits (Table 1). Similarly, a significant mean square due to doubled haploid lines vs checks was also observed. These results suggested the existence of wide variability among the maize doubled haploid lines for all the traits under study. Non-significance of mean squares due to blocks indicated the poor evidence for a detectable effect of edaphic factors and / or micro-environments associated with the blocks for all the traits.

Box and Whisker Plots

Box plot is the way to represent range of numerical data of several traits by constructing box and whisker plots (Tukey, 1977). A box plot can provide information about a sample's range, median, normality of the distribution and skewness of the distribution (Kumar *et al.*, 2019). It can also identify and plot extreme cases within the sample. The distribution properties of seven quantitative parameters estimated in doubled haploid population derived from crossing CV-29073758 × CV-29073679 are presented in box plots (Fig.1) depicting the degree of dispersion in the population. Bold lines within boxes indicate median value and box limits indicate interquartile range (*i.e.*, 50% of values lie within the box) and whiskers indicate highest and lowest excluding outliers or extremes. In our data set, among seven traits, only cob length, number of kernel rows per cob and cob girth showed very few outliers as indicated by the points outside the whisker.

Test for Skewness and Kurtosis

The results of phenotypic statistics which includes mean, skewness, kurtosis, minimum, maximum are shown in Table 2. The genetic expectation of coefficient of skewness for the distribution of DH lines for plant height, ear height and cob length was positively skewed (Table 2, Fig. 2). Positive skewness indicated the involvement of complementary gene

TABLE 1

Analysis of variance for seven quantitative traits in 275 maize doubled haploid lines

Source	df	Plant height (cm)	Ear height (cm)	Cob length (cm)	Cob girth (cm)	Number of kernel rows/ cob	Kernel weight / cob (g)	Test weight (g)
Blocks	13	178.8 ns	53.53 ns	2.1 ns	0.16 ns	7.56 ns	192 ns	5.72 ns
Checks	2	4657.14 **	1616.67 **	35.41 **	26.60 *	43.76 ns	921.56 *	1832.55 **
Doubled haploids	274	442.9 **	143.23 **	19.28 *	22.65 **	63.09 *	1007.69 **	21.77 **
Doubled haploids vs checks	272	411.92 **	132.4 **	25.34 **	20.27 *	52.12 ns	1008.32 **	20.87 **
Error	26	61.63	33.33	5.12	0.29	4.37	186.06	7.05

ns-non-significant; *, **significant at P<0.05, P<0.01

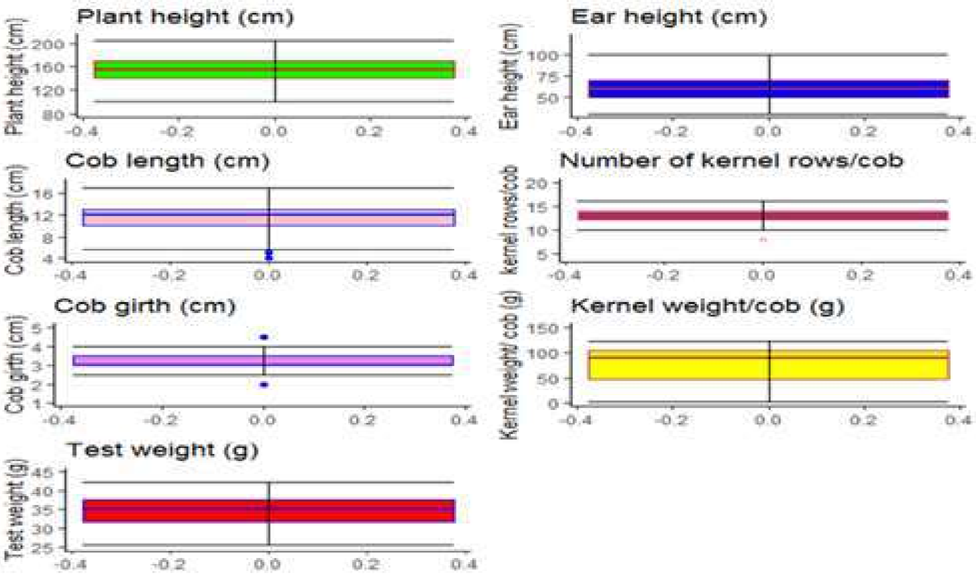

Fig. 1: Box plot depicting the distribution properties of seven quantitative traits in maize doubled haploid population

interactions predominantly in the same direction (Snape and Riggs, 1975).

Similarly, all the quantitative traits exhibited platykurtic distribution (<3.0). It indicated the involvement of many genes, with the majority of them displaying complementary epistasis with increasing effects (Table 2, Fig. 2). Thus, expected genetic gain in such a condition would be slow under mild selection while it is rapid with intense selection. From these statistical values, it was concluded that the seven quantitative traits were controlled by multiple genes. Whereas, platykurtic and negatively skewed distribution recorded for cob length, number of kernel rows per cob, kernel weight per cob and test weight revealed large number of genes displaying duplicate dominant epistasis. Sumathi *et al.* (2018) also has reported positive skewness for cob diameter, 100 grain weight and grain yield per plant in Maize.

Correlation Studies for Yield and Yield Component Traits in Doubled Haploid Population

Selection based on the correlation studies gives an idea about magnitude and direction of association between yield and its attributes. Phenotypic correlations between kernel weight per cob and

TABLE 2

Descriptive statistics on seven quantitative traits in maize doubled haploid lines

Traits	Mean	Std. Error	Skewness	Kurtosis	Median	Min	Max	Variance
Plant height (cm)	153.4234	1.278	0.0494	-0.1127	155	100	205	445.9668
Ear height (cm)	59.9088	0.765	0.238	-0.1383	60	30	100	160.1194
Cob girth (cm)	3.1989	0.033	0.1794	-0.3335	3	2	4.5	0.3154
Cob length (cm)	11.6204	0.143	-0.4034	0.0307	12	4	16	5.637
Number of kernel rows per cob	12.9781	0.11	-0.4545	-0.1313	14	8	16	3.4229
Kernel weight per cob (g)	75.56	1.99	-0.605	-1.013	89.6	1.9	122.7	1089.91
Test weight (g)	34.5599	0.22	-0.2829	-0.5937	35.025	25.6	42.33	14.3694

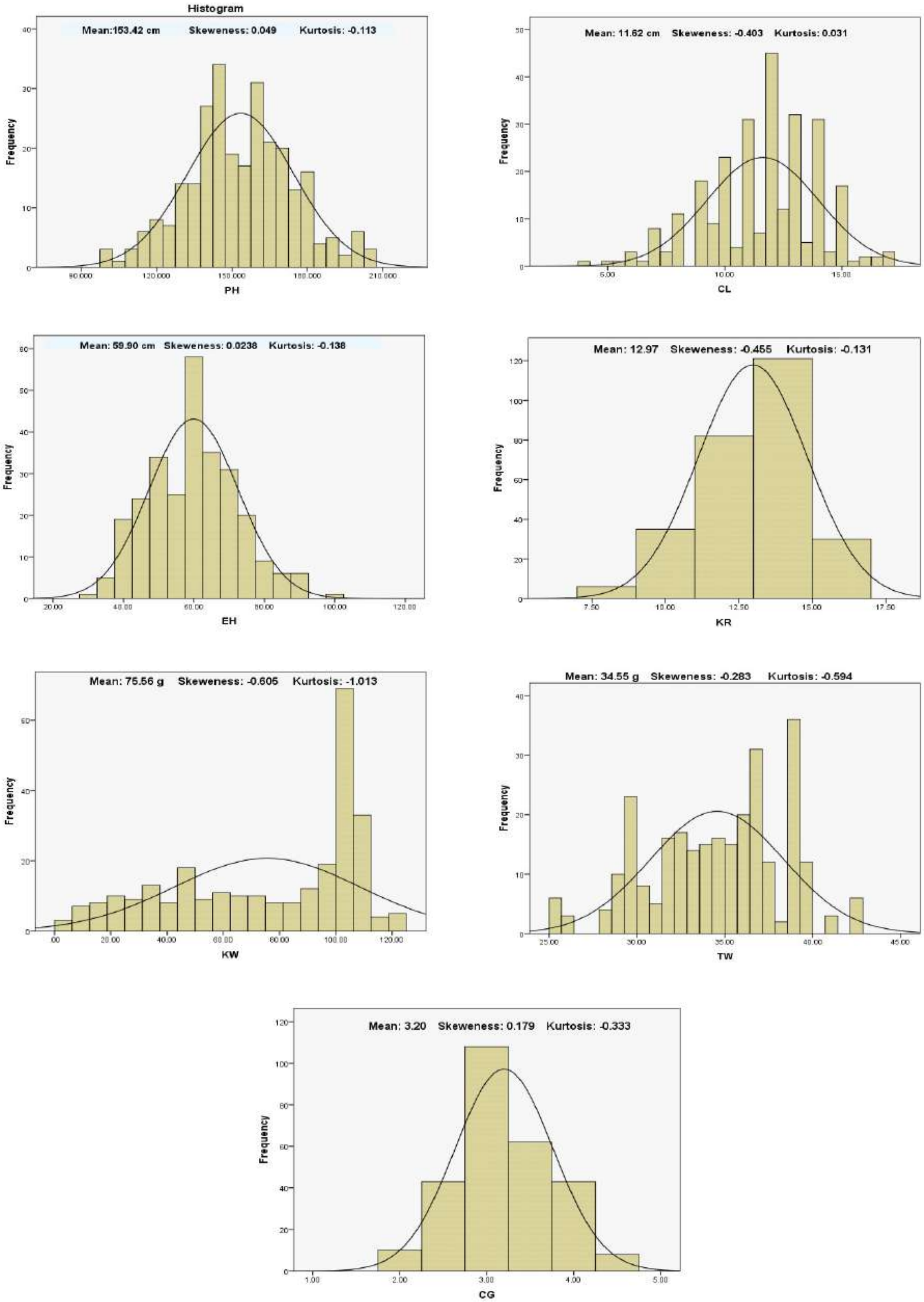

Fig.2: Frequency distribution of seven quantitative traits in maize doubled haploid lines

yield components *viz*., plant height; ear height, cob girth, cob length, number of kernel rows per cob, kernel weight per cob and test weight were computed for doubled haploid population and the results are presented in Fig. 3 & 4. Kernel weight per cob had a significant positive correlation with cob length, cob girth, kernel rows per cob ($p<0.001$) followed by plant height ($p<0.01$), and ear height ($p<0.05$). It indicated that kernel weight per cob can be increased through improvement in characters that showed positive and significant association. Similar results were reported earlier in maize by several workers on different characters *viz*., for the association of kernel yield with plant height (Raghu *et al*., 2011 and Zarei *et al*., 2012), ear height (Raghu *et al*., 2011 and Munawar *et al*., 2013), number of kernel rows per cob (Sofi and Rather, 2007) and 100 grain weight (Raghu *et al*., 2011, Nataraj *et al*., 2014 and Zarei *et al*., 2012).

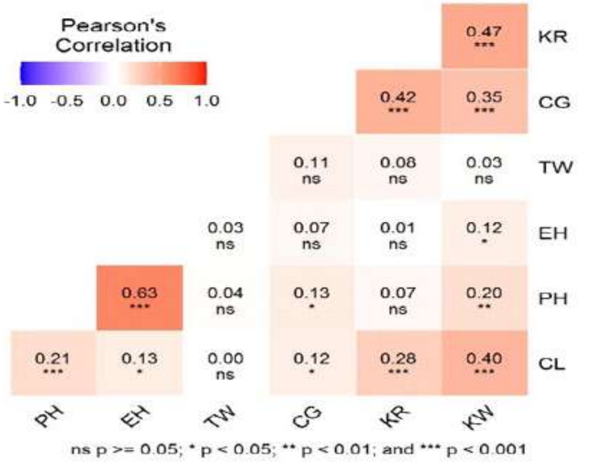

Fig. 3: Pearson correlations among seven morphological traits in doubled haploid population in maize (*Zea mays* L.)

Principal Component Analysis (PCA)

PCA was performed on the data recorded on seven quantitative characters in 275 maize DH lines. Out of seven principal components (PCs) three exhibited more than 1.00 eigen value and showed about 67.22 per cent cumulative variability among the traits studied. Eigen values of three principal component axes and percentage of variation obtained from the principal component analysis are presented in Table 3. The PC1 exhibited 30.87 per cent while, PC2 and PC3 exhibited 21.25 per cent and 15.10 per cent variability, respectively among the doubled haploids for the traits under study.

Individual Trait Contribution to Total Germplasm Variability

Starting with the first PC, the plot sloped steeply downward initially and then became approximately horizontal line. The point at which the curve first began to straighten out was considered to indicate the maximum number of components indicated in Fig.5. (Kumawat *et al*., 2021)

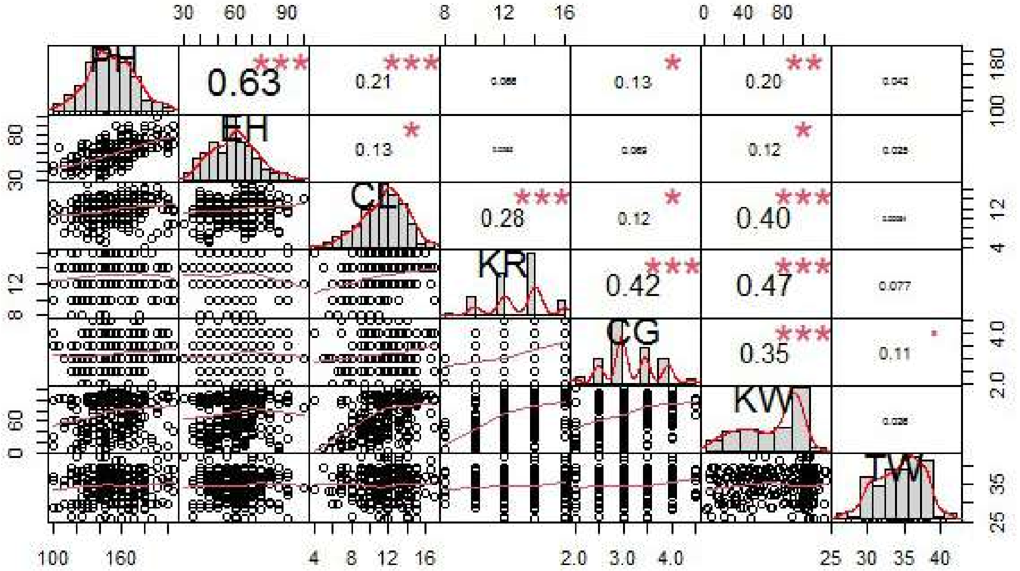

Fig. 4: Scatter plot with correlation values of seven quantitative traits in doubled haploid population in maize (*Zea mays* L.)

TABLE 3

Per cent variability explained by the seven eigen vectors

Parameters	PC1	PC2	PC3
Eigen values	2.16	1.487	1.057
% Variance explained	30.86	21.24	15.10
cumulative % variance	30.86	52.11	67.22
Plant height	15.22	32.05	0.09
Ear height	10.06	39.32	0.22
Cob length	15.78	0.34	9.51
Cob girth	15.60	7.00	9.24
Kernel rows per cob	20.13	15.53	0.45
Kernel weight per cob	22.67	5.69	5.91
Test weight	0.49	0.03	4.55

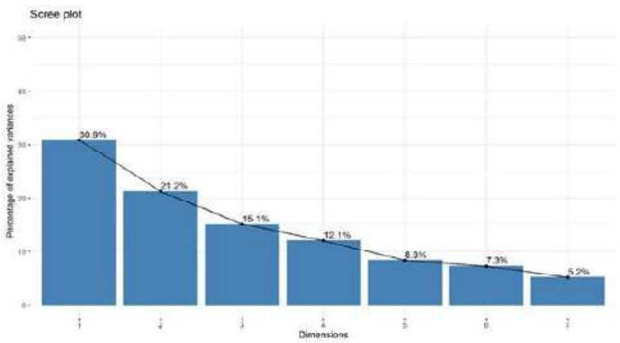

Fig. 5: Scree plot illustrating eigen values corresponding to seven extracted principal components

It could be seen from the first vector that the characters plant height (15.22), ear height (10.07), cob length (15.78), cob girth (15.61), kernel rows per cob (22.67), kernel weight per cob (22.67) and test weight (0.49) displayed positive contribution towards genetic divergence. The characters that contributed to the second component include plant height (32.05), ear height (39.32), cob length (0.35), cob girth (7.00), kernel rows per cob (15.53), kernel weight per cob (5.69) and test weight (0.03). PC III accounted 15.14 per cent of the total variability. The traits plant height (0.09), ear height (0.22), cob length (9.51), cob girth (9.24), kernel rows per cob (0.45), kernel weight per cob (5.91) and test weight (4.55) displayed positive contribution towards genetic diversity. Maji & Shaibu (2012) used PCA to categorize traits of maize (*Zea mays* L.) that contributed for most of the variance in the data.

The biplot diagram between PC 1 and PC 2 explained the distribution and the nature of diversity for both variables and the genotypes (Fig. 6). The genotypes which are close to the origin contributed less to the total genetic variance, whereas those genotypes away from the origin contributed more to the total variance.

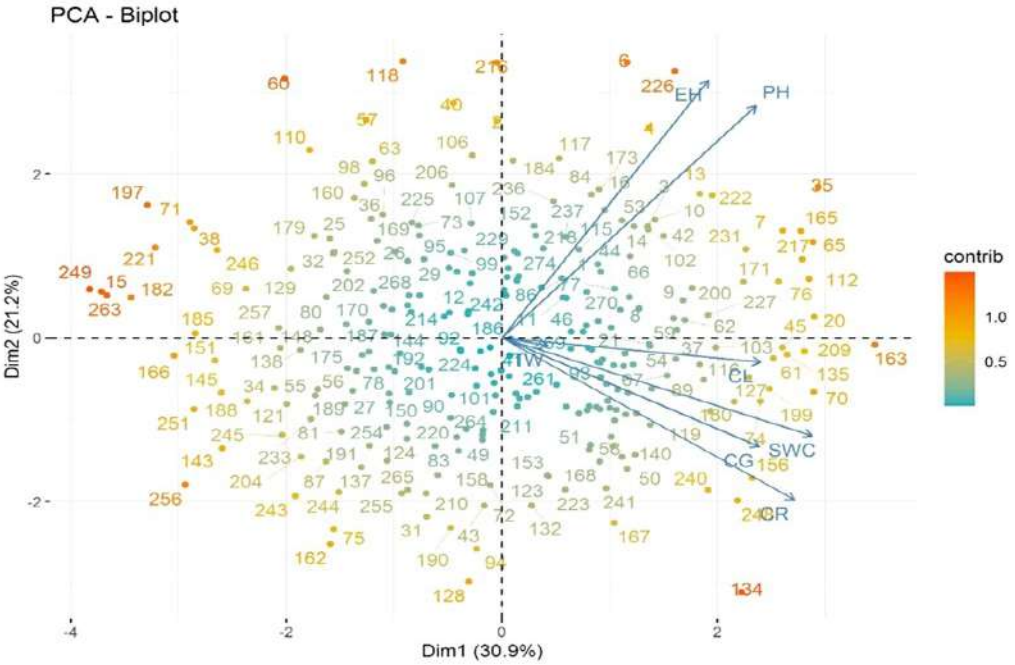

Fig. 6 : A genotype by trait bi-plot representing 275 doubled haploid maize lines distribution

Similar kind of observation was made by Swapnil *et al*. (2021). The traits plant height and ear height were found to be positively associated (an acute angle) and they were independent of cob length, cob girth, number of kernel rows per cob, kernel weight per cob and test weight. The bi-plot diagram exhibited high variability among the genotypes and between the parameters. (Yugandhar *et al*., 2018).

The K-mean clustering grouped the 275 DH lines in to three clusters (Table 4, Fig. 7). Cluster-C comprised of 32 doubled haploid inbred lines which showed maximum plant height (175.65), ear height (73.59), cob girth (3.51), cob length (12.53), kernel weight per cob (92.37) and test weight (35.64). Cluster-B consisted of 118 inbred lines with similar performance for morphological trait *i.e.* number of kernel rows per cob (13.77). Cluster-A had 110 genotypes characterized by the features such as the lowest plant height (145.64), ear height (56.06), cob girth (2.92), cob length (10.43), kernel weight per cob (59.65) and test weight (33.95). Since cluster C consisted of lines superior for kernel weight and other major productivity traits, these DH lines can be used in the development of superior hybrids.

TABLE 4

Cluster Mean of doubled haploid inbred lines included in three different K-clusters

Traits	Cluster A	Cluster B	Cluster C
PH (cm)	145.64	153.19	175.65
EH (cm)	56.06	59.27	73.59
CG (cm)	2.92	3.4	3.51
CL (cm)	10.43	12.36	12.53
KR	11.96	13.77	13.31
KW (g)	59.65	83.64	92.37
TW (g)	33.95	34.5	35.64

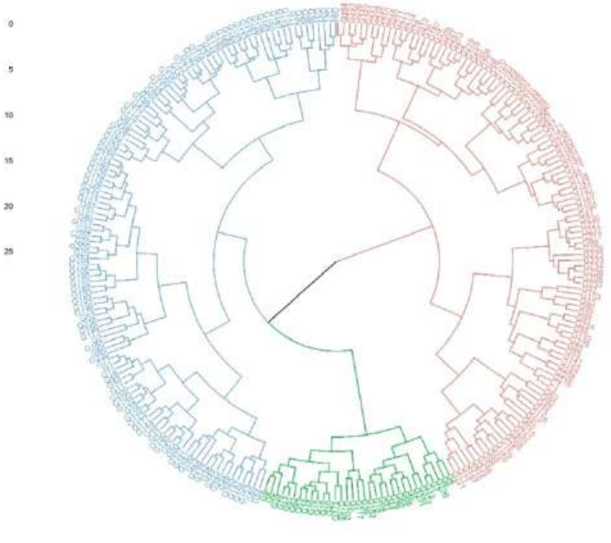

Fig. 7: K- means clustering of 275 doubled haploid lines

All the quantitative traits exhibited platykurtic distribution (< 3.0). It indicated the involvement of many genes, with the majority of them displaying complementary epistasis with increasing effects. The present investigation of PCA analysis revealed that the genotypes which are close to the origin in biplot, contributed less to the total genetic variance whereas those genotypes away from the origin contributed more to the total variance and also recorded better *per se* performance for the plant height, EH, CL, CG, KR, KW and TW(expand the terms). So, these genotypes could be used to develop heterotic hybrids in future maize breeding programme. Kernel weight per cob had a significant positive correlation with cob length, cob girth, kernel rows per cob followed by plant height and ear height. It indicated that kernel weight per cob can be increased through improvement in characters that showed positive and significant association. By K-means clustering its concluded that cluster C consisted of lines superior for kernel weight and other major productivity traits, these DH lines can be used in the development of superior hybrids.

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