

Full Length Research Article

Association and Variability Analysis of Indigenously Developed Maize Inbred Lines Under Drought Stress at Vegetative Stage

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Abstract

“Maize is an important cereal that is much sensitive to drought stress throughout its life cycle, this stress has serious impacts on its morphological and phenological attributes. Direct selection for yield under stress conditions may be unfruitful, so selection for yield contributing traits is implied. Present study was conducted to evaluate indigenously developed inbred lines and to assess the strength of correlations between different yield contributing traits. Inbred lines IB-13, IB-43, IB-35 were found drought tolerant and are positively related with yield contributing traits like number of rows (NOR), grains per row (GPR), 100 grain weight (100GW), total grains (TG) while inbred lines IB-47, IB-20, IB-19, IB-48, IB-16, IB-34, and IB-9 were performing low for yield related traits thus found drought susceptible. Cob length (CL), cob diameter (CD), number of rows (NOR) and grains per row (GRP) showed significant positive correlation with total grains (TG). Cluster analysis was performed to assess the variability in the inbred lines and five clusters were made according to their relatedness. Moreover, parameters like TG, GPR, 100GW, NOR followed by CL, NOC with high GAM values can be used as selection standard for better selection purposes. These selected inbred lines can be used in future breeding programs.

Keywords: Maize, Drought Stress, Selection, Correlation, Cluster Analysis, Principal Component Analysis

Introduction

Maize is the third most important cereal crop after Wheat and Rice, having major contribution in food, feed, bio-fuel and serves as raw material for many processes. Crop production is intensively affected due to extreme variation in environmental factors such as temperature fluctuation, sea level elevation and variation in precipitation. Nevertheless water deficiency, salinity, heat, and cold are most devastating abiotic stresses that adversely limit agricultural production leading to immense yield losses (Aslam et al., 2014; Ishaq et al., 2015; Singh et al., 2019). Among major environmental concerns that limit the growth, development and production potential of the plants is low water availability. ‘Drought’ and ‘water deficit stress’ are two terminologies that are mostly used to reflect the availability of water to the plants, though both are regarded as ‘soil moisture stress’ and it varies with physiochemical attributes of the soil. Drought results in physiological impairment of the plant functions due to inadequate water supply to the plant, leading to

senescence, while water deficit cause growth retardation of the plants due to lack of sufficient availability of water for absorption and transport of solutes from soil for optimum plant growth and development (Meseka et al., 2018; Tandzi et al., 2019).

Water is an essential component of actively growing plants, constitute 70-90% of fresh biomass. Plant structure, growth as well as morphological and physiological functions are severely affected under water stress, results in immense yield loss. Water stress causes production of reactive oxygen species (ROS) that results in protein denaturation (Moharramnejad et al., 2019). Water requirement of the crops vary in various tissues, growth stages of plants from seedling to maturation and among different plants species as well (Jain et al., 2015). Response of the plants vary with period of exposure, intensity of stress, plant growth stage and tolerance level.

Wheat, rice and maize are major cereals and important component of human diet. Together, they are accounted for about 42.5 % food calorie supply of the world. Maize is also a major staple crop, grown under

wide range of climatic condition. It is also essential nutritional and economic crop globally (W. Li et al., 2018). Water requirement of maize is more at reproductive growth stage as compared to early developmental stage. In maize during peak vegetative development period about 28-32% loss of dry weight occur under short duration water deficit condition whereas 66-93% yield loss expected to occur as a result of prolong water stress (Cakir, 2011). Grain yield of maize is also sensitive to water deficit. Under abiotic stress kernel number is more precisely correlated with grain yield than weight of kernels. Depending upon the intensity and time of water deficit kernel number can be reduced up to 100%. Loss of kernel number results from inhibited silk emergence and incomplete floret development (Wang et al., 2018). Short term water deficit condition delays the leaf tip emergence while long term water deficit reduced the final size of leaves, internodes and 15-52% yield loss. Size reduction of leaves affects the photosynthesis as leaves are the photosynthetic factory of the plants (Cakir, 2011). More than 90% yield reduction occur at tasseling and silking stage due to water deficiency. Almost 70% grain yield loss occur under intermediate water stress while 90% grain yield under severe water stress condition. Leaf area index, ear number and yield reduction occur under water deficit condition and it also affects the quality parameters such as protein and sugar contents. Crude protein contents decreased from 18% to 12% under water stress (Aydinsakir et al., 2013). Alteration in metabolic path ways, stomatal closure, reduced nutrients and CO₂ uptake will also take place (Shafiq et al., 2019). It also markedly affects the photosynthesis because of reduction in photosynthetic pigments that significantly reduced the assimilation of carbon dioxide.

Understanding of complex genetics of drought tolerance is pre-requisite to breed drought tolerant varieties. Present study was conducted for evaluating indigenously developed inbred lines for their variability because it is imperative for a breeder to collect variable genotypes before starting any breeding program. Moreover, direct selection for yield is not very useful so we use correlation analysis to determine the relationship between yield and its contributing traits and for identification of traits which can be used for selection of drought tolerant genotypes. Cluster and Principal Component Analysis was done which can aid in identification of drought tolerant genotypes. (Dao et al., 2017; Gazal et al., 2018; Ishaq et al., 2015; Mehri, 2015)

Material and Methods:

Climatic Conditions

Present study was conducted under the climate of semi-arid type where irrigation plays vital role in agricultural productivity. Normally, weekly irrigation of maize is mandatory for good yield. In spring, when temperature reaches above 35°C, weekly irrigation becomes extremely necessary. Delayed irrigation has serious impacts on yield of crop (Meseka et al., 2018).

Experimental Conditions

Sowing was done following randomized complete block design during spring season of 2018 with row to row and plant to plant distances maintained at 9cm and 75cm respectively. After 45 days when plants were at 7th to 8th leaf stage, irrigations were given after 15 days interval till 14th leaf stage when tip of the tassel was starting to appear (Bell, 2017). At reproductive stage, maize was irrigated normally to precisely evaluate the effect of water stress at vegetative stage. Standard cultural practices were followed from to harvesting

Plant Material

Indigenously developed 48 inbred lines (i.e. IB1, IB2, IB3, IB4.....IB46, IB47, IB48) were used, which were developed thorough self-pollination for successive generations of selected plants (till 6 generations) from diverse germplasm, which was obtained from different research stations.

Growth Parameters

Total 12 characters were recorded to study the response of plants i.e. Plant height (PH), Ear Height(EH), Ear leaf width (ELW), Days to 50% anthesis (DA), Days to 50% silking (DS), Cob length(CL), Cob Diameter (CD), Number of cobs (NOC), Number of rows per cob (NOR), Grains per row (GPR), Total grains (TG), 100 grain weight(100GW). PH, EH and ELW were measured after emergence of the tassel leaf with measuring tape and scale. PH was measured by measuring tape from soil surface to base of tassel while ear height was measured from soil surface to base of lowermost ear. DA was recorded from sowing to days to 50% anthesis and DS was recorded when 50% plants of each genotype started silking. CD was measured using vernier-caliper while CL was measured by standard scale. 100 GW was measured on electrical balance (grains were dried till 15% moisture content). Remaining characters were counted manually.

Statistical Analysis

Collected data was subjected to analysis of variance through Statistical Software STAR. Phenotypic, Genotypic, Environmental variances, Phenotypic Coefficient of Variation, Genotypic Coefficient of Variation, Heritability and Genetic Advance was calculated according to (Ahsan et al., 2015; Pinnisch et al., 2012).

Result and Discussions

Variability Analysis

Analysis of variance was done through statistical software STAR. Table [1] shows mean square and CV values for all 12 characters. Characters such as PH, EH, ELW, DA, DS, CL, CD, NOR, GPR, TG and 100 GW were found highly significant i.e. $P < 0.01$ while NOC was significant i.e. < 0.05 . Similar significant results were obtained by (Saeed et al., 2018; Tandzi et al., 2019). Highest CV value was observed for NOC i.e. 17.41 while low value of CV for other characters show reliability of this experiment. Values for Phenotypic Variance (PV), Genotypic Variance (GV), Environmental Variance (EV), Phenotypic Coefficient of Variation (PCV), Genotypic Coefficient of Variation (GCV), Heritability (h^2) and Genetic Advance as function of Mean (GAM) was calculated according to (Falconer, 1989), is given in [Table 2]. PCV and GCV values were high for GPR, TG, NOC and 100 GW with little difference between both values showing high contribution of genetic variations rather than the influence of the environment, which indicates that these traits can be used for effective selection of drought tolerance genotypes. Heritability ranged from 91.047041 to 99.933954, highest heritability was observed for TG and lowest for CL, whereas the value of GAM was as low as 12.79 for DA and as high as 141.94 for TG. High GAM value for TG, GPR, 100GW, NOR followed by CL, NOC shows that these characters with combination of heritability can be used as potential indicators for selection against water deficit conditions as heritability can't be used alone for selection purposes as it is influenced by both phenotypic and genotypic variances. These findings were in accordance with (Ahsan et al., 2015; Aminu & Izge, 2012; Gazal et al., 2017; Mustafa et al., 2013).

Cluster Analysis

Variability is pre-requisite of any breeding program. Figure [1] is showing variability in mean values of all observed characters for studied inbred

lines. Cluster analysis was performed for 48 inbred lines [Table 3] and five clusters were made, which was latterly confirmed by dendrogram [Figure 2], clearly depicting five different clusters of inbred lines. Inbred lines of cluster 2 and 4 can be used for selection of yield parameters like NOR, 100GW, NOC while for early maturity selection inbred lines of cluster group 5 can be used. Saeed et al. (2018) used cluster analysis to assess 70 genotypes and made three distinct clusters of genotypes with variable characteristics. Genotypes of a cluster are more closely related as compared to genotypes of other clusters. Table [3] shows cluster groups while cophene correlation coefficient (shows strength of a pairing model) value is 0.553, which shows weakness of this pairing model. More the value closer to 1 more will be the accuracy of cluster analysis. Group 2 containing 25 genotypes showing little contribution of these genotypes in overall exhibited variability. These findings were in similarity with findings of (Rafique et al., 2018; Shrestha, 2016; Suryanarayana et al., 2017).

Correlation Analysis

Grain yield is a complex quantitative character, so knowledge of its association with other characters will aid in plant breeding programs (Premlatha & Kalamani, 2010). Correlation analysis was done for all 12 characters [Table 4] and it was found that all characters were positively correlated to each other except, NOC which was negatively correlated to DA (-0.0469) and 100GW (-0.1857). CD was negatively correlated to PH (-0.026), NOR was negatively correlated to ELW (-0.0931), EH (-0.156) and PH (-0.0771) while 100GW was negatively correlated with PH (-0.0187), DA (-0.031). [Fig 3] is showing scatter plot matrix that shows correlation among different variables. Kumar et al. (2017) found that grains rows per cob, grains per row, ear diameter and 100 kernel weight are all yield contributing traits. Strong positive correlation [Table 4] of cob length (0.3878**), cob diameter (0.6342**), number of rows (0.6974**) and grains per row (0.9091**) with total grains show that these traits can be targeted for breeding of high yield. This was in accordance with (Effendi et al., 2019; Gazal et al., 2018; Mustafa et al., 2013; Nemati et al., 2009; Premlatha & Kalamani, 2010).

Principal Component Analysis (PCA)

High degree of correlation between different variables makes PCA very effective for analysis. Principal Component Analysis was performed and variation was divided into 12 principal components. Four principal components have eigen value of more

than 1[Table 5] but analysis was performed for three principal components i.e. PC1, PC2 and PC3[Figure 4, 4.1, 4.2] which are accounting for 65.59% of total variability. Numerical representation of eigen values explains how variability influence the dependence structure of all variables (Aslam, 2014; Saeed et al., 2018). This information was derived from scree test [Figure 5] which gives us information that how much principal components should be retained in an exploratory analysis. Variability exhibited by genotypes is clear from depicted biplots. Kravić et al. (2016) found that number of kernels per row, grain yield and leaf width are most differentiating traits among genotypes in PCA. Genotypes that are far from the origin are better performers under water stressed conditions while genotypes falling on opposite direction are drought susceptible. According to Biplots Inbred lines 13, 43, 35, 22, 25 and 41 are better performers as they are more linked to yield determining traits as compared to others while inbred lines 47, 20, 19, 48, 16, 34 and 9 are susceptible to water stress conditions. Vectors for all yield related characters are in positive quadrant and are showing strong correlation among them, while vectors for vegetative characters and for DA, DS are falling in fourth quadrant. Inbred lines 43, 21, 25, 37, 17, 18, 26, 13, 14, 23, 11 and 22 are positively related with CL, CD, NOR, GPR, TG, 100 GW while Inbred lines 5, 16, 25, 28, 38 and 48 are linked with days to anthesis and days to silking, so these genotypes can be used to breed long or short duration hybrids. These results were in accordance with (Ali, Ahsan, Ali and Kanwal, 2017; Aminu and Izge, 2012; Dao et al., 2017; Gazal et al., 2018; Pinnisch et al., 2012).

Conclusion

It is concluded that sufficient variability was exhibited by all inbred lines as can be seen from cluster analysis and principal component analysis. Based on cluster characteristics effective selection of inbred lines can be made. IB 43, IB 21, IB 25 and IB 37 can be used as potential inbred lines for breeding high yielding varieties under water deficit conditions or can be used in any breeding program. Heritability accompanied with genetic advance can be used for effective selection purposes. High GAM value for TG, GPR, 100GW, NOR followed by CL, NOC shows that these characters can be used as potential indicators for selection against water deficit conditions.

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Table1: Analysis of Variance

Source	Df	PH	EH	ELW	DA	DS	NOC	CL	CD	NOR	GPR	TG	100GW
Blocks	2	13.0588	3.64	0.4672	0.5278	1.2153	0.13	3.29	1.4847	0.1372	1.2123	4.64	0.6829
Genotypes	47	1160.011*	225.09**	3.11**	49.4604**	72.9689**	0.8048*	19.7607**	10.9639**	40.5505**	159.89**	26236.308**	153.7499**
Error	94	23.5766	6.9	0.2327	0.3505	0.3571	0.0468	1.7756	0.5614	0.8251	1.08	17.27	0.2554
CV		5.18	8.93	9.46	0.8983	0.8258	17.41	14.58	7.52	9.36	8.71	3.045	2.61

* = significant at 5% probability level, ** = significant at 1% probability level

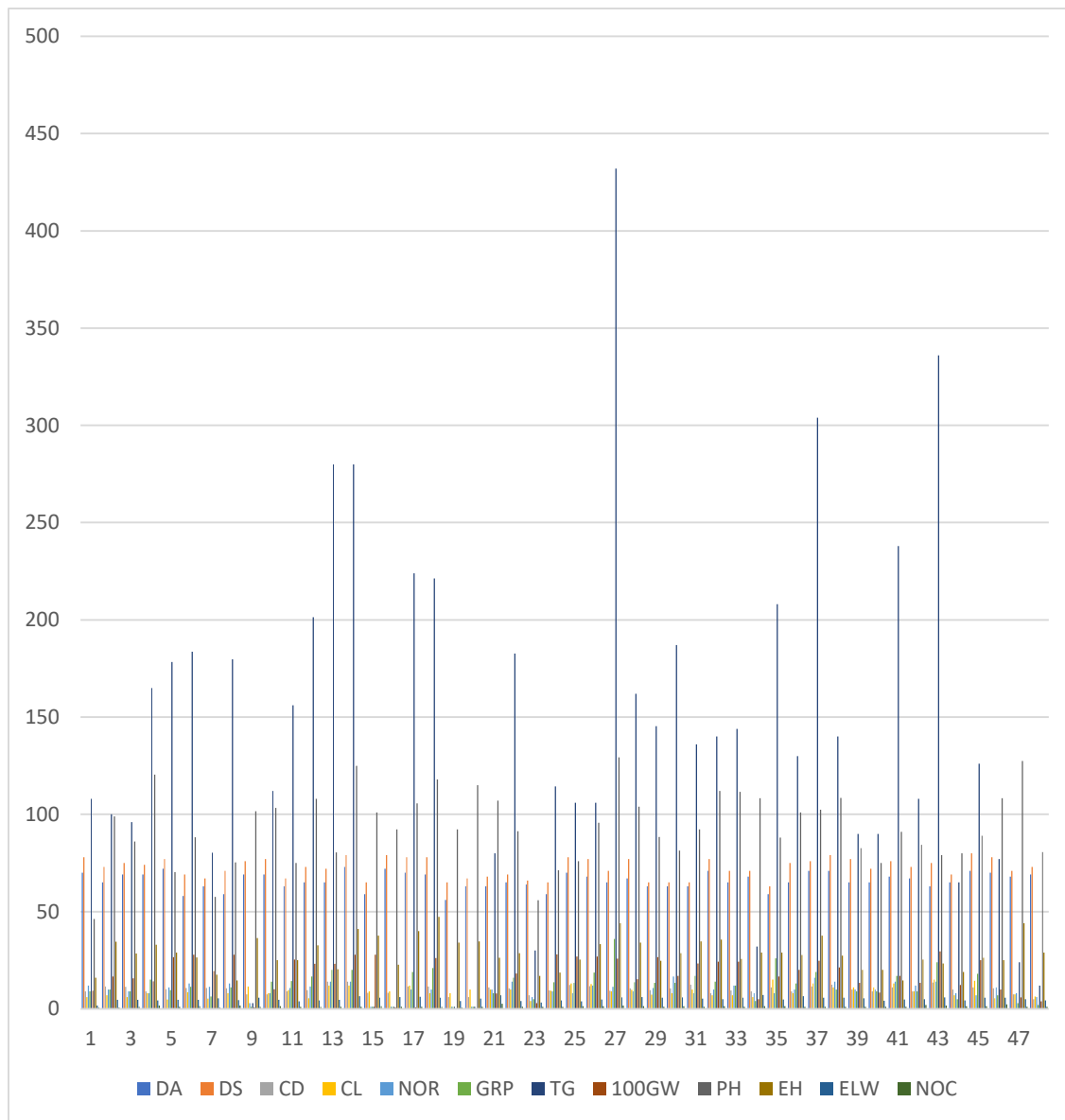
Df=Degree of Freedom, PH=Plant Height, EH=Ear Height, ELW=Ear Leaf Width, DA=Days to Anthesis, DS=Days to silking, NOC=Number of Cobs, CL=Cob Length, CD=Cob Diameter, NOR=Number of Rows, GRP=Grains per Row, TG=Total Grains, 100GW=100 Grain Weight, CV=Coefficient of Variation

Table 2: Variability, Heritability and Expected Genetic Advance of all Characters

TRAIT	P.V	G.V	E.V	P.C.V	G.C.V	h ²	GAM
PH	386.67	378.81	7.86	21.05	20.84	97.967259	40.05
EH	75.03	72.73	2.3	30.33	29.87	96.93456	55.73
ELW	1.04	0.96	0.078	20.04	19.25	92.307692	37.19
DA	16.49	16.36	0.3	6.15	6.13	99.211643	12.79
DS	24.32	24.2	0.122	6.6	6.78	99.506579	13.94
NOC	0.2683	0.253	0.0156	43.38	42.13	94.297428	64.1
CL	6.59	6	0.59	27.57	26.82	91.047041	56.52
CD	3.65	3.4675	0.1871	19.2	18.72	95	39.06
NOR	13.51	13.24	0.28	37.93	37.55	98.00148	78.27
GPR	53.3	52.93	0.37	60.39	60.74	99.305816	123.55
TG	8745.436	8739.66	5.776	67.67	68.85	99.933954	141.94
100GW	51.25	51.16	0.09	42.06	42.02	99.82439	113.15

P. V=Phenotypic Variance, G. V=Genotypic Variance, E. V=Environmental Variance, P.C. V=Phenotypic Coefficient of Variation, G.C. V=Genotypic Coefficient of Variation, h²=Heritability, GWM=Genetic Advance as Function of Mean

Figure 1: Histogram showing mean values of all characters



Df=Degree of Freedom, PH=Plant Height, EH=Ear Height, ELW=Ear Leaf Width, DA=Days to Anthesis ,DS=Days to silking, NOC=Number of Cobs, CL=Cob Length, CD=Cob Diameter, NOR=Number of Rows ,GRP=Grains per Row, TG=Total Grains, 100GW=100 Grain Weight

Table 3: Cluster Groups with their Characteristics

Cluster	Cluster Size	Cluster Inbred	Cluster Major Traits (Maximum)	Cluster Major Traits (Minimum)
1	3	IB1 IB23 IB48		CL, PH, ELW
2	27	IB2 IB3 IB5 IB6 IB7 IB8 IB11 IB12 IB13 IB22 IB24 IB25 IB26 IB29 IB30 IB31 IB33 IB35 IB36 IB38 IB39 IB40 IB41 IB42 IB43 IB44 IB45	DS, CD, CL, NOR	DS,100GW, EH
3	9	IB4 IB9 IB10 IB16 IB21 IB32 IB34 IB46 IB47	ELW	GPR, TG, NOC
4	6	IB14 IB17 IB18 IB27 IB28 IB37	DA, GPR, TG, PH, EH	
5	3	IB15 IB19 IB20		DA, CD, NOR,100GW, NOC

IB=Inbred Line, PH=Plant Height, EH=Ear Height, ELW=Ear Leaf Width, DA=Days to Anthesis ,DS=Days to silking, NOC=Number of Cobs, CL=Cob Length, CD=Cob Diameter, NOR=Number of Rows ,GRP=Grains per Row, TG=Total Grains, 100GW=100 Grain Weight

Figure 2: Dendrogram showing clustering of all forty-eight genotypes

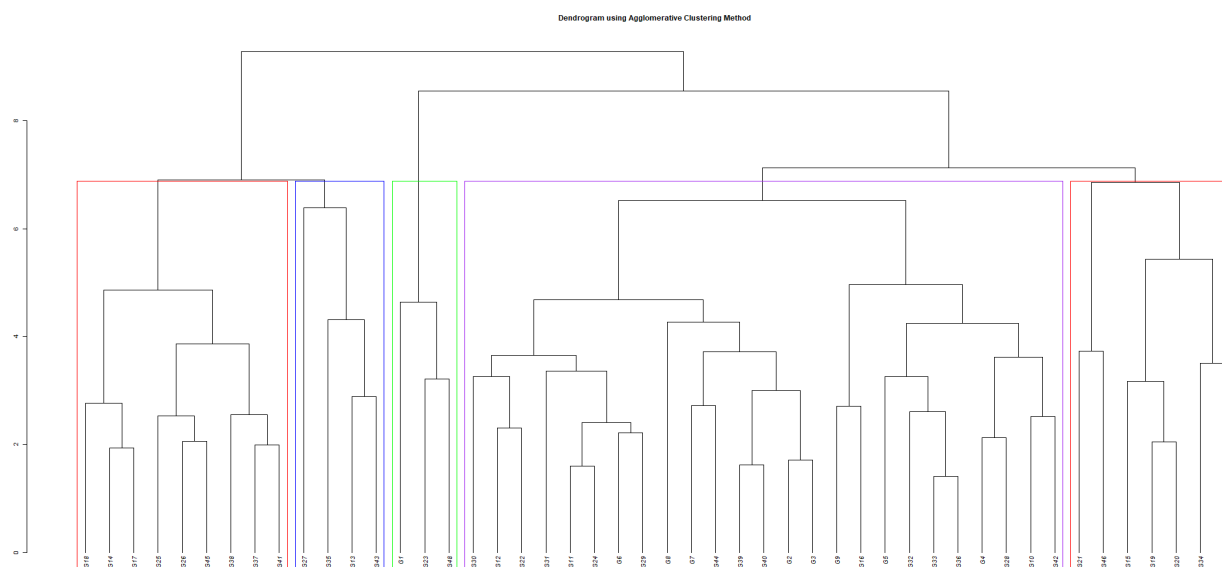


Table 4: Correlation Analysis

	PH	EH	ELW	DA	DS	NOC	CL	CD	NOR	GPR	TG	100GW
PH	1											
EH	0.7687**	1										
ELW	0.6417**	0.4402*	1									
DA	0.2605	0.2947*	0.1028	1								
DS	0.2237*	0.2449*	0.0702	0.8397**	1							
NOC	0.1982*	0.0158	0.2	-0.0469	0.0244	1						
CL	0.119	0.158	0.2171	0.0334	0.1723	0.0093	1					
CD	-0.026	0.0223	0.1434	0.1387	0.2295*	0.1179	0.4917**	1				
NOR	-0.0771	-0.156	-0.0931	0.129	0.1592	0.1132	0.1158	0.6240**	1			
GPR	0.1381	0.2524	0.0333	0.0383	0.135	0.0797	0.4646**	0.5986**	0.5668**	1		
TG	0.1062	0.1682	0.0538	0.0638	0.1754	0.0607	0.3878**	0.6342**	0.6974**	0.9091**	1	
100GW	-0.0187	0.1058	0.1919	-0.031	0.1208	-0.1857	0.369**	0.4684**	0.1942	0.4491**	0.4828**	1

PH=Plant Height, EH=Ear Height, ELW=Ear Leaf Width, DA=Days to Anthesis, DS=Days to silking, NOC=Number of Cobs, CL=Cob Length, CD=Cob Diameter, NOR=Number of Rows, GRP=Grains per Row, TG=Total Grains, 100GW=100 Grain Weight

Figure 3: Scatter Plot Matrix

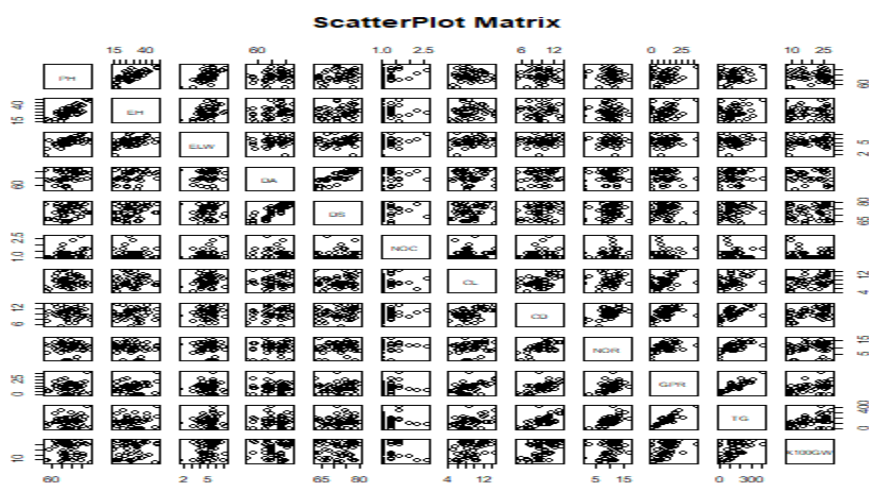


Table 5: Variability exhibited by all Principal Components

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
Eigen Values	3.7926	2.4604	1.6175	1.2172	0.8847	0.6675	0.5153	0.3138	0.2145	0.1384	0.1149	0.0631
Variability %	31.6	52.11	13.48	10.14	7.37	5.56	4.29	2.61	1.79	1.15	0.96	0.53
Cumulative	31.6	20.5	65.59	75.73	83.1	88.67	92.96	95.57	97.36	98.52	99.47	1

PC=Principal Component

Figure 4: PCA1 and PC2 showing Genotypes and Variables.

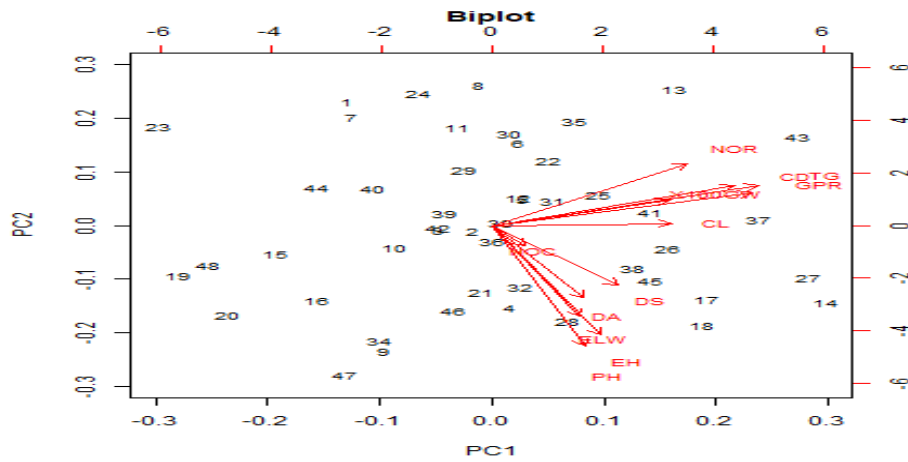


Figure 4.1: PCA1 and PCA3 showing Genotypes and Variables

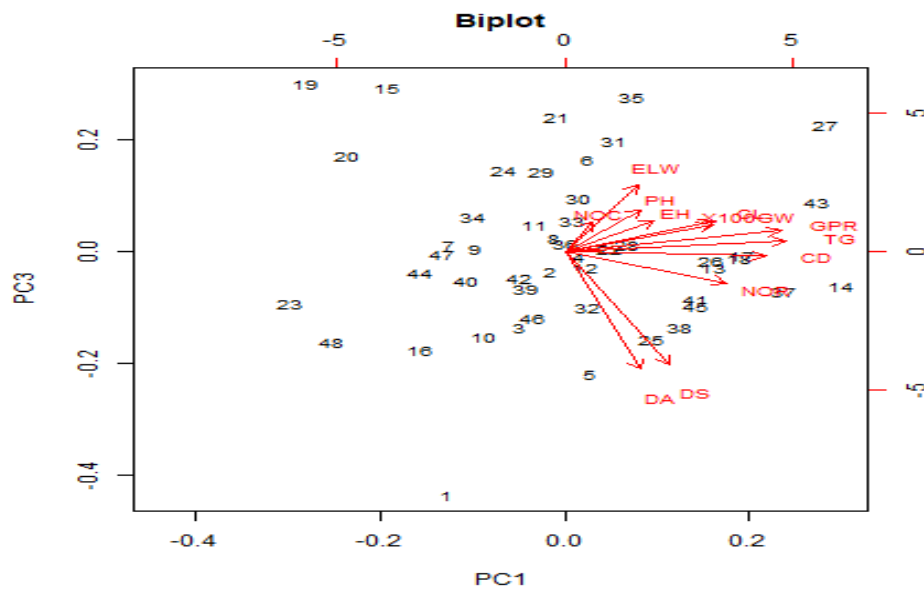


Figure 4.2: PCA2 and PCA3 showing Genotypes and Variables.

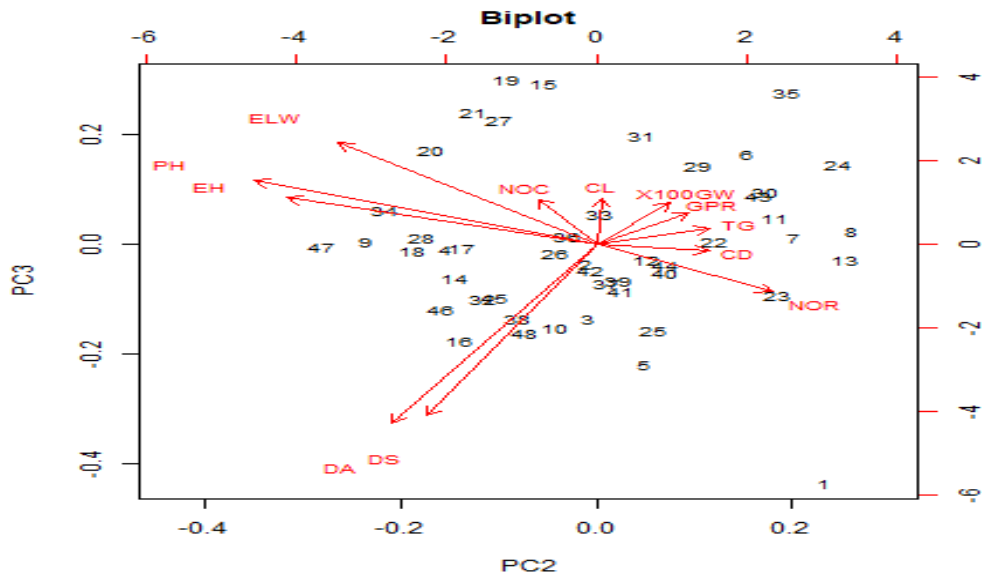


Figure 5: Scree Plot depicting all the principal components

