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Genetic Variability and Divergence Studies in Maize (*Zea mays* L.) Inbreds under Moisture Stress Condition

Mani B. R.
Ph.D. Scholar, Department of GPB, College of Agriculture, U.A.S., Dharwad, Karnataka, India
Deshpande S. K.
Professor and Head, Department of GPB, College of Agriculture, U.A.S., Dharwad, Karnataka, India

Abstract:
The above study was conducted during summer 2015, at Department of Genetics and Plant Breeding, UAS, Dharwad. Aim of the experiment was, to assess the amount of genetic variability and divergence found in ninety- eight maize inbreds under induced moisture stress situation in comparison with four tolerant checks. Occurrence of wide range of statistically significant variability has been confirmed by the analysis of variance. Traits associated with moisture stress tolerance such as, ASI, Chlorophyll content, RWC and yield parameters had exhibited high heritability combined with high GAM thus, selection based on these parameters could be recommended to improve stress tolerance. ASI, PHT, CHT, CLN and kernel rows per cob exhibited Positive and significant correlations with grain yield in stress. While, Kernels per row followed by Shelling percentage and Fodder yield per plant had showed direct and positive effect on grain yield in stress environment. Diversity analysis using Mahalanobi's statistics, has clustered the study material into eight clusters of which Cluster I was the largest with fifty-five inbreds and Cluster V, VII and VIII were solitary, inferring the existence of wide range of diversity in the materials considered for the study. Large inter cluster distance was observed between the Cluster IV and Cluster VI suggesting crossing between the members of such clusters to exploit maximum heterosis in terms moisture stress tolerance. Among the eight different clusters, mean values favorable to enhance yield in stress environment were found in Cluster II for the traits ASI, CWT, KRPC and SP and Cluster V for PHT, CLN, CGR and kernels per row. Lines from these two clusters can be selected for improvement of respective traits. The current study has found out the prevalence of huge variability and diversity required for initiating any crop improvement programme. Therefore, it would be recommended to utilize some of the better inbreds like PDM 6529, PDM4641, PDM6549, PDM6563, PDM 6541 and few others in enhancing stress tolerance.

Keywords: *Diversity, maize, moisture stress, path coefficients, variability, yield*

1. Introduction

Maize (*Zea mays* L.)is one of the most important cereal crop of the world, India as well as Karnataka. It is being utilized as food for human and feed for livestock as well as ingredient for more than five hundred food products thus, has great yield demand. To meet the requirements of such industries, productivity of the crop has to be high but various abiotic stresses reduce the grain yields. Among all such constrains, drought or moisture stress remains the most important devastating factor. Soil moisture deficit during critical crop growth stages may cause drastic yield reduction, especially if it occurs during the reproductive phase (Basseti and Westgate, 1993).

Chlorophyll content is one of the major factors affecting photosynthetic capacity. Reduction or no-change inchlorophyll content of plant under drought stress has been observed in different plant species and its intensity depends on stress rate and duration (Jagtap et al 1998). Generally, it seems that osmo- regulation is one of the main mechanisms preserving turger pressure in most plant species against water loss, it causes plant to continue water absorption and retain metabolic activities thus, amount of water retained under stress is referred to as, Relative Water Content (RWC) (Gunasekera and Berkowiz, 1992). Therefore, breeding for drought tolerant maize cultivars or SCH's, will likely to increase maize production beyond the present level. Progress in plant breeding depends on the extent of genetic variability and diversity present in the population. Hence, the first step in any breeding program is the study of the genetic variability and divergence present in breeding material.

Progress from selection has been reported to be directly related to the magnitude of genetic variance in the population (Tabanao and Bernardo, 2005). Similarly, determining the genetic diversity is an essential first step for identifying suitable parents for hybridization. Obviously the diverse parents are expected to through higher frequency of heterotic hybrids besides generating a broad spectrum of variability in segregating generations. The objective of the present study was therefore, to estimate genetic variability and diversity of maize inbred lines under induced water stress environment. Such information will help in identification of lines that could be used to develop drought-tolerant maize varieties, synthetics and Single Cross Hybrids (SCH).

2. Material and Methods

The present study consisted of ninety-eight inbreds and four tolerant inbreds, evaluated by following Randomized Block Design with two replications at Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwada, Karnataka, during *Summer* 2015. Moisture stress was induced at taseling stage and continued till the harvest with single protective irrigation after fifteen days of taseling. Observations on five randomly selected plants from each line were collected related to yield and drought tolerant traits such as Days Fifty per cent Taseling (DFT), Days to Fifty per cent Silking (DFS), Anthesis to Silking Interval (ASI), Plant Height (PHT), Cob height (CHT), Cob weight (CWT), Cob Length (CLN), Cob Girth (CGR), Kernals Rows Per Cob (KRPC), Kernals Per Row (KPR), Test Weight (TW), Shelling Percentage (SP), Grain Yield per Plant (GYP), Fodder Yield per Plant (FYP) and Harvest Index (HI). Vernier caliper, electronic balance and measuring scale were used to estimate CGR, CWT and PHT respectively. Secondary or physiological traits such as, Chlorophyll content and Relative Water Content (RWC) of third leaf from top during ninety days after sowing were recorded using SPAD 502 meter and Barr and Weatherley 1962, method respectively. Mean values were considered for analysis using WINDOSTAT 8.0software. Genetic variability feature like, mean, range, Genetic Variability (GV), Phenotypic Variability (PV), Genetic and phenotypic coefficient of variation (GCV and PCV), Heritability in broad since (h^2), Genetic advance over percent Mean (GAM) and character associations were calculated. Similarly, Genetic divergence parameters such as construction of clusters, calculation of cluster means, determination of intra and inter cluster distance and percent contribution of individual traits towards the divergence among the inbreds were estimated according to Singh and Chaudhary (1985) and drawn the following results.

3. Results and Discussion

Analysis of variance (Table 1) had showed the occurrence of statistically significant variation across the inbreds based on, seventeen different primary and secondary traits considered in the study. Results depicted in Table 2 draws following inference. Anthesis to Silking Interval (ASI) is the most important trait that determines drought tolerance. It showed a mean of 3.01 with a range of 1-11. It has exhibited high PCV and GCV (58.76; 44.71) with narrow range inferring least influence of environment on it. It was also assessed from table 2 that, ASI had 57.90% heritability (broad sense) coupled with 70.09 GAM. These results were in confirmation with Saleem et al2011. Chlorophyll content and RWC were also a common feature in enhancing moisture stress tolerance, these two parameters exhibited wide range of variability along with high heritability and GAM (88.50% and 93.30%) as well as high mean (36.39 and 85.67) across the inbreds studied, these results are similar with results of Ganji et al 2012. Similarly, Cob weight (78.60% and 69.23) and kernels per row (60.50 and 30.36)showed high h^2 and GAM. High heritability coupled with high genetic advance give high response to selection or effective on the basis of ASI, Chlorophyll content, RWC, Cob weight and Kernels per row under moisture stress condition.

Grain yield is a quantitative trait, as it is controlled by many genetic and environmental factors. Information on genotypic correlation coefficients among various traits is therefore, useful to ascertain the degree to which these are associated with economic productivity. It was clear from Table 3, traits such as ASI (0.30**), PHT (0.70**), CLN (0.88**), KPR (0.87**) and CGR (0.74**) had positive and significant association with grain yield in moisture stress situation therefore, indirect selection based on these traits would improve yield under stress. These findings were supported by the findings of Hussain et al 2009. Days to 50% Taseling, silking and RWC exhibited positive and non-significant association with grain yield in moisture stress. Dissociation of correlations into direct and indirect effects and provides the information on actual contribution of a trait on the yield. Table 4 give us such information saying that, Kernels Per Row showed highest positive direct effect (0.472), followed by Shelling Percentage (0.336) and Test Weight (0.024). Path coefficient analysis also confirmed that, 99.29% of variation observed in the study is explained by genotypes and only 0.0843 is due to residual effect that is some other factors also affecting grain yield.

The genetic diversity analysis facilitates stratified sampling of breeding population and helps in identification of genotypes for hybridization. Classification of ninety-eight inbreds and four checks led to the formation of eight clusters(Fig 1). The cluster I consisted of highest (55) inbreds, followed by cluster II (16) and cluster III with fifteen inbreds. Cluster V, VII and VIII were singleton clusters. The genotypes falling in the same cluster are more closely related and possess narrow genetic diversity. Genotypes of singletons are highly unique in traits considered. Genetic distance between the lines determine their performance in hybrid combinations, thus from the Table 5 it was clear that, between cluster IV and VI wide genetic distance exist (13.41), followed by cluster VI and Cluster VIII (13.00) and Cluster VI and VII (12.84).

Source of Variation	df	DFT	DFS	ASI	PHT	CHT	CWT	CLN	CGR	KRPC
Replication	1	10.83	25.41	3.06	159.60	177.20	2.67	0.06	0.22	6.46
Treatment	101	14.91**	18.72**	4.96*	1137.79**	506.05**	2535.00	11.68**	0.33*	5.54*
Error	101	1.81	2.49	1.32	319.00	176.57	303.04	5.55	0.10	1.61
SEM		0.95	1.11	0.81	12.57	9.35	12.25	1.66	0.22	0.89
CV		2.04	2.29	38.13	10.71	18.86	19.75	17.60	8.48	9.44
CD (5%)		2.67	3.13	2.28	35.43	26.36	34.53	4.67	0.61	2.52

Source of Variation	df	KPR	TW	SP	FYP	HI	CHL (90 DAS)	RWC (90DAS)	GYPP (MS)
Replication	1	27.24	378.38	80.24	59.75	2.88	391.59	44.02	8.15
Treatment	101	53.77**	34.86**	331.90**	93.23**	4.66*	121.85**	166.29**	1697.32**
Error	101	13.25	15.18	157.80	39.51	1.93	7.46	5.81	82.17
SEM		2.56	2.74	8.84	4.42	0.59	1.92	1.70	6.38
CV		15.33	17.54	18.96	28.72	28.75	7.39	2.81	15.52
CD (5%)		7.22	7.73	24.92	12.45	1.65	5.42	4.78	17.98

*Table 1: Analysis of variance for the various moisture stress and yield related parameters in maize inbreds at full grain maturity stage Where * and ** indicate significant at 5% and 1% of LOS.*

Genetic parameters	DFT	DFS	ASI	PHT	CHT	CWT	CLN	CGR	KRPC	KPR	TW	SP	FYP	HI	CHLO	RWC	GYPP
Mean	66.01	69.01	3.01	166.78	70.46	88.16	13.40	3.65	13.44	23.75	22.21	66.26	21.88	3.52	36.93	85.67	58.40
GV	6.55	8.12	1.82	409.39	164.74	1115.98	3.067	0.12	1.97	20.26	9.84	87.051	26.86	1.37	57.20	80.24	807.58
GCV	3.88	4.13	44.71	12.13	18.22	37.89	13.089	9.47	10.44	18.96	14.12	14.08	23.68	33.21	20.48	10.46	48.66
PV	8.36	10.61	3.14	728.39	341.31	1419.02	8.614	0.22	3.58	33.51	25.02	244.85	66.37	3.30	64.65	86.05	889.74
PCV	4.38	4.72	58.76	16.18	26.22	42.731	21.94	12.71	14.08	24.38	22.52	23.61	37.23	51.62	21.77	10.83	51.08
h^2	0.78	0.77	0.58	0.56	0.48	0.786	0.36	0.56	0.55	0.61	0.39	0.36	0.41	0.41	0.89	0.93	0.91
GAM	7.07	7.44	70.08	18.74	26.07	69.227	16.09	14.53	15.94	30.36	18.24	17.30	31.03	44.03	39.68	20.80	95.50

Table 2: Genetic variability parameters for yield and moisture stress related characters in maize inbreds at full grain maturity stage

Where,

DFT- Days to fifty percent flowering; DFS-Days to fifty percent silking; ASI-anthesis to silking interval; PHT- Plant height (cm); CHT- Cob height (cm); CWT- Cob weight (g); CLN- Cob length (cm); CGR- Cob girth (cm^2); NKPC- Number of kernel rows per cob; NKPR- Number of kernels per row; TW-Test weight (g); SP-Shelling percentage (%); FYP- Fodder yield per plant (g); HI-Harvest index; CHLO-Chlorophyll content; RWC- Relative Water Content; GYPP- Grain Yield Per Plant(g);

	DFT	DFS	ASI	PHT-	CHT	CWT	CLN	CGR	KRPC	KPR	TW	SP	FYP	HI	CHLO	RWC	GYPP
DFT	**1.0**	0.88*	-0.04	-0.70*	-0.56*	-0.54*	-0.34*	-0.46*	-0.50*	-0.60*	-0.15	-0.25*	-0.13	0.02	0.01	-0.10	-0.51*
DFS		**1.0**	0.44*	-0.69*	-0.48*	-0.56*	-0.46*	-0.59*	-0.57*	-0.63*	-0.29*	-0.49*	-0.11	-0.10	-0.16	-0.05	-.01
ASI			**1.0**	-0.13	0.04	-0.17	-0.33*	-0.37*	-0.25*	-0.19	-0.32*	-0.56*	0.03	-0.25*	-0.37*	0.08	0.30*
PHT				**1.0**	0.87*	0.7*	0.58*	0.63*	0.46*	0.74*	0.48*	0.48*	0.34*	-0.12	0.08	0.05	0.70*
CHT					**1.0**	0.67*	0.37*	0.43*	0.36*	0.65*	0.31*	0.51*	0.32*	-0.21	0.08	0.07	0.70*
CWT						**1.0**	0.88*	0.73*	0.61*	0.87*	0.65*	0.47*	0.37*	-0.18	0.28*	0.03	0.98*
CLN							**1.0**	0.58*	0.58*	0.89*	0.57*	0.47*	0.30*	-0.28*	0.28*	0.06	0.88*
CGR								**1.0**	0.84*	0.63*	0.66*	0.41*	0.23	-0.01	0.21	-0.05	0.74*
KRPC									**1.0**	0.73*	0.01	0.33*	0.20	-0.02	0.16	-0.09	0.61*
KPR										**1.0**	0.42*	0.47*	0.28*	-0.14	0.19	-0.01	0.87*
TW											**1.0**	0.40*	0.02	0.09	0.18	0.20	0.69*
SP												**1.0**	0.08	0.34*	0.15	-0.03	0.64*
FYP													**1.0**	-0.81*	0.40*	0.01	0.33*
HI														**1.0**	-0.30*	0.02	0.10
CHLO															**1.0**	-0.01	0.24*
RWC																**1.0**	0.01
GYPP																	**1**

Table 3: Correlation coefficients among moisture stress and yield related traits at full grain maturity stage

Clusters means shown in Table 6 deduce that, inbred line of cluster V (PDM 6529) has very low mean ASI (1.00), followed by cluster II (2.31) and cluster VI (PDM 4641, PDM 6549, PDM6563) with 2.17 mean ASI. Inbred, PDM 6541 of cluster VIII had very high chlorophyll content and RWC (45.42 and 90.13%). Inbreds of cluster II also had high mean values with respect to cob weight (142.98), kernel rows per cob (14.48) and shelling percentage (74.33%). Similarly mean values for plant height (203.50), cob length (16.85), cob girth (3.87) and kernels per row (31.80) were high in cluster V. Thus, lines from these two clusters (V and II) are effective source for improvement of drought tolerance. Relative Water Content (32.58%) followed by Chlorophyll content (21.59%) and Shelling percentage (17.71%) were the major traits contributed highest towards diversity observed in the present study (Fig 2). Based on foregoing results and discussion, it can be suggested that, Inbreds such as, PDM 6529, PDM4641, PDM6549, PDM6563 and PDM 6541 as well as all sixteen inbreds of cluster II were found to possess all the desirable traits necessary to be a moisture stress tolerant lines. Utilization of such lines in crop improvement in terms of drought tolerance would give expectable results.

	PHT	CHT	CWT	CLN	CGR	KRPC	KPR	TW	SP	FYP	CHLO	RWC
PHT	**-0.0082**	-0.0072	-0.0061	-0.0048	-0.0052	-0.0038	-0.0061	-0.0039	-0.0040	-0.0028	-0.0007	-0.0004
CHT	-0.2870	**-0.3308**	-0.2200	-0.1230	-0.1415	-0.1190	-0.2157	-0.1028	-0.1691	-0.1070	-0.0265	-0.0227
CWT	0.8686	0.7851	**1.1807**	1.0431	0.8588	0.7163	1.0328	0.7698	0.5560	0.4411	0.3317	0.0407
CLN	-0.3292	-0.2121	-0.5040	**-0.5705**	-0.3292	-0.3335	-0.5095	-0.3258	-0.2703	-0.1700	-0.1576	-0.0360
CGR	-0.0120	-0.0081	-0.0138	-0.0109	**-0.0189**	-0.0159	-0.0119	-0.0125	-0.0077	-0.0043	-0.0040	0.0010
KRPC	-0.0418	-0.0324	-0.0547	-0.0527	-0.0756	**-0.0902**	-0.0654	-0.0011	-0.0301	-0.0183	-0.0142	0.0079
KPR	0.3495	0.3076	0.4126	0.4212	0.2956	0.3421	**0.4717**	0.1992	0.2234	0.1338	0.0910	-0.0024
TW	0.0112	0.0073	0.0153	0.0134	0.0155	0.0003	0.0099	**0.0235**	0.0095	0.0005	0.0043	0.0046
SP	0.1626	0.1720	0.1584	0.1594	0.1370	0.1124	0.1593	0.1358	**0.3364**	0.0271	0.0494	-0.0104
FYP	0.0186	0.0174	0.0201	0.0160	0.0122	0.0109	0.0152	0.0011	0.0043	**0.0537**	0.0216	0.0003
CHLO	-0.0040	-0.0040	-0.0140	-0.0138	-0.0106	-0.0078	-0.0096	-0.0091	-0.0073	-0.0200	**-0.0499**	0.0005
RWC	0.0015	0.0022	0.0011	0.0020	-0.0016	-0.0028	-0.0002	0.0062	-0.0010	0.0002	-0.0003	**0.0316**
GYPP	0.7295	0.6969	0.9756	0.8795	0.7364	0.6090	0.8706	0.6804	0.6401	0.3338	0.2448	0.0147
Partial R^2	-0.0060	-0.2306	1.1519	-0.5017	-0.0139	-0.0549	0.4107	0.0160	0.2153	0.0179	-0.0122	0.0005

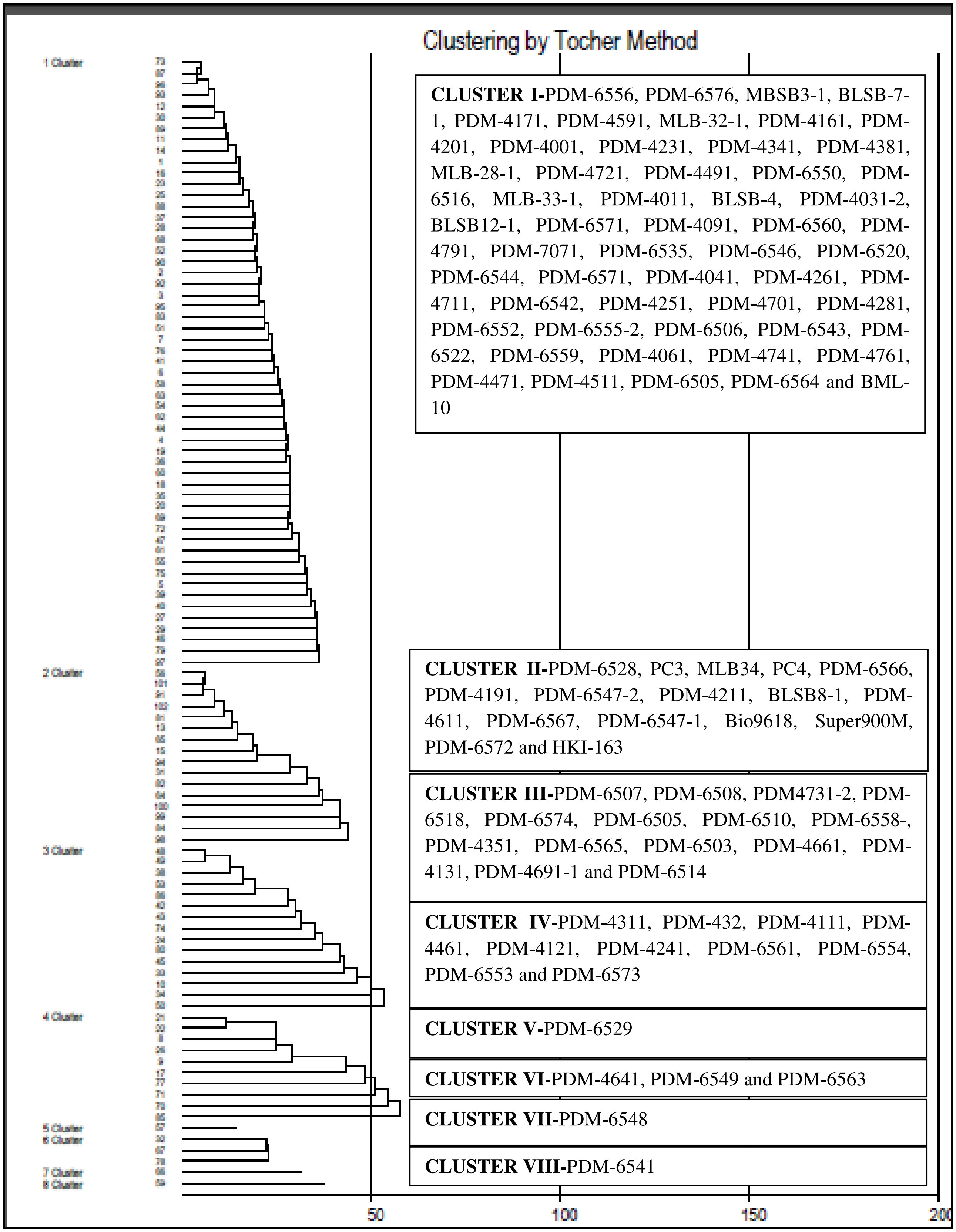
Table 4: Direct (diagnol) and indirect (off diagnol) effects of independent variables on dependent variable at full grain maturity stage R square = 0.9929; Residual effect = 0.0843

	Group. 1	Group. 2	Group. 3	Group. 4	Group. 5	Group. 6	Group. 7	Group. 8
Group. 1	**5.35**	7.74	7.04	7.60	8.72	11.84	7.22	8.11
Group. 2		**5.84**	9.22	10.46	8.34	12.72	8.23	8.17
Group. 3			**6.61**	9.22	8.49	9.21	8.65	9.22
Group. 4				**7.30**	9.87	13.41	8.98	10.73
Group. 5					**0.00**	8.18	9.98	10.19
Group. 6						**5.50**	12.84	13.00
Group. 7							**0.00**	7.94
Group.8								**0.0**

Table 5: Inter (diagnol) and intra (above diagnol) cluster distances of maize inbreds

	DFT	DFS	ASI	PHT	CHT	CWT	CLN	CGR	KRPC	KPR	TW	SP	FYP	HI	CHLO	RWC	GYPP
Group.1	65.96	68.82	2.85	165.12	68.58	77.61	12.95	3.60	13.33	22.72	21.32	66.04	22.92	3.33	37.17	88.35	49.82
Group.2	64.00	66.31	2.31	190.19	82.69	142.98	16.27	4.08	14.48	30.26	27.66	74.33	22.63	3.57	41.47	90.07	105.66
Group.3	67.70	70.60	2.90	156.52	62.07	78.01	12.70	3.64	13.66	22.07	20.40	58.41	19.51	3.47	40.50	76.79	44.78
Group.4	66.40	71.90	5.50	152.58	65.10	61.86	11.77	3.35	12.62	21.52	21.05	63.01	16.05	4.81	23.10	87.25	39.09
Group.5	63.00	64.00	1.00	203.50	98.50	140.00	16.85	3.87	13.60	31.80	27.50	69.15	20.00	3.46	24.93	71.22	96.50
Group.6	67.50	69.67	2.17	164.33	70.33	96.33	14.31	3.68	13.18	24.50	23.00	72.02	22.50	3.42	37.82	54.13	69.33
Group.7	69.50	73.50	4.00	139.50	48.50	106.00	11.00	2.55	8.00	13.00	20.50	68.25	39.00	1.75	36.57	89.99	72.00
Group.8	67.00	70.50	3.50	177.50	151.80	112.00	12.92	3.42	13.20	23.90	17.50	77.69	30.00	2.92	45.42	90.13	87.00

Table 6: Cluster means for the various yield and stress tolerant traits of maize inbreds



CLUSTER I-PDM-6556, PDM-6576, MBSB3-1, BLSB-7-1, PDM-4171, PDM-4591, MLB-32-1, PDM-4161, PDM-4201, PDM-4001, PDM-4231, PDM-4341, PDM-4381, MLB-28-1, PDM-4721, PDM-4491, PDM-6550, PDM-6516, MLB-33-1, PDM-4011, BLSB-4, PDM-4031-2, BLSB12-1, PDM-6571, PDM-4091, PDM-6560, PDM-4791, PDM-7071, PDM-6535, PDM-6546, PDM-6520, PDM-6544, PDM-6571, PDM-4041, PDM-4261, PDM-4711, PDM-6542, PDM-4251, PDM-4701, PDM-4281, PDM-6552, PDM-6555-2, PDM-6506, PDM-6543, PDM-6522, PDM-6559, PDM-4061, PDM-4741, PDM-4761, PDM-4471, PDM-4511, PDM-6505, PDM-6564 and BML-10

CLUSTER II-PDM-6528, PC3, MLB34, PC4, PDM-6566, PDM-4191, PDM-6547-2, PDM-4211, BLSB8-1, PDM-4611, PDM-6567, PDM-6547-1, Bio9618, Super900M, PDM-6572 and HKI-163

CLUSTER III-PDM-6507, PDM-6508, PDM4731-2, PDM-6518, PDM-6574, PDM-6505, PDM-6510, PDM-6558-, PDM-4351, PDM-6565, PDM-6503, PDM-4661, PDM-4131, PDM-4691-1 and PDM-6514

CLUSTER IV-PDM-4311, PDM-432, PDM-4111, PDM-4461, PDM-4121, PDM-4241, PDM-6561, PDM-6554, PDM-6553 and PDM-6573

CLUSTER V-PDM-6529

CLUSTER VI-PDM-4641, PDM-6549 and PDM-6563

CLUSTER VII-PDM-6548

CLUSTER VIII-PDM-6541

Figure 1: Clustering pattern of ninety-eight maize inbreds and four checks based on Touchers' method

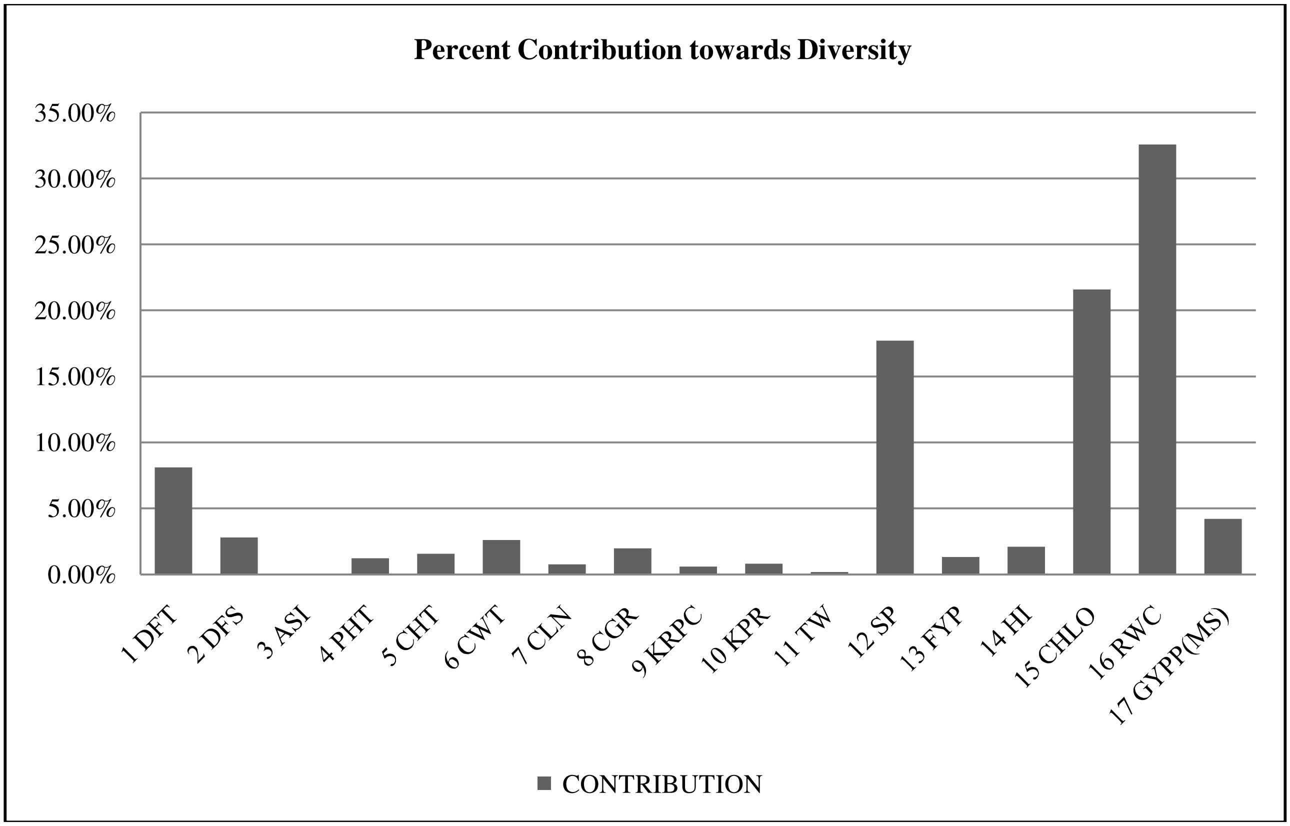


Figure 2: Characters sharing percent contribution to the total diversity observed in the maize inbred lines

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