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Original Research Article

Genetic Diversity under Heat Stress Condition in Maize (*Zea mays* L.) Inbred Lines

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Keywords

Cluster analysis, D^2 statistics, genetic diversity, heat stress, inbred line

A B S T R A C T

The experiment was conducted to understand genetic diversity in 29 CIMMYT maize inbred lines of diverse origin under normal and heat stress condition for thirteen yield and yield attributing characters at Maize Section of Bihar Agricultural College of Bihar Agricultural University, Sabour, Bhagalpur, Bihar (India). In normal condition, D^2 statistics displayed that Cluster IV, being the largest group, comprises 8 maize inbred lines followed by cluster V, VI, I and III having seven, six, four and three inbred lines, respectively, while Cluster II having mono genotype cluster. Under heat stress condition, all the inbred lines were also grouped into six clusters, with cluster I, V and VI containing the maximum of six inbred lines followed by five inbred lines in cluster III and three inbred lines each in cluster II and IV. In normal condition, the maximum intra-cluster distance was obtained for cluster III (34.23) followed by cluster II (25.91) and, cluster I (18.42), whereas, the maximum inter-cluster distance was recorded between cluster II and VI (221.41) followed by cluster IV and VI (145.24) and cluster III and VI (129.82). Under heat stress condition the intra-cluster distance was maximum in cluster III (76.02) followed by cluster II (71.35) and cluster I (42.06), whereas, the highest inter cluster distance was observed between cluster IV and VI (464.04) followed by cluster III and VI (382.83), cluster V and VI (318.92). The plant height (39.90%) showed maximum contribution towards total genetic diversity followed by 500-grainswight (23.89%) and grains per plant (16.01%) in normal condition, whereas, under heat stress condition, the maximum contribution towards total genetic diversity was contributed by 500-seed weight (24.38%) followed by plant height (17.98%) and shelling % (17.24%) Therefore, these characters may be given due importance in hybridization programme. Considering cluster distance, inter-genotypic distance, mean values and other agronomic performances the inbred lines, namely, CML304, CML 305 and CML306 from cluster IV and CML18, CML161, CML172, CML189, CML308, CML451 from cluster VI may be considered as better parents for future hybridization programs to obtain desirable heat tolerant maize hybrids.

Introduction

Maize (*Zea mays* L.) is considered as a queen of cereals and par excellence in terms of food, feed, fodder, bio-fuel and industrial raw materials. In India, maize is being grown on an area of 8.69 million hectares with annual production of 21.81 million tonnes and average grain yield of 2509 kg ha^{-1} (Anonymous, 2016). In Bihar, maize is being grown on an area of 0.70 million hectares with annual production of 2.40

million tonnes and average grain yield of 3416 kg ha^{-1} (Anonymous, 2016). Maize is being grown during three seasons i.e. *kharif*, *rabi* and spring in Bihar. India's population is projected to continue growing for several decades from 1.34 billion in 2017 to around 1.5 billion in 2030 and approaching 1.66 billion in 2050 (Anonymous, 2017). Diets are projected to continue to become more affluent and the need for maize as animal feed is expected to increase as more of the population becomes able to afford eggs, milk and meals (Msangi and Rosegrant, 2011). Closing the "yield gap" (the difference between observed and optimal yields) in developing regions has the potential to increase global maize production by 45% (Gustafson *et al.*, 2014). The increasing food and feed demand, coupled with the fluctuation in temperature and rainfall patterns as a consequence of climate change, a major challenge is to optimize crop production. Heat in itself is a profitable and natural resource but becomes dangerous and poses harmful effects, when it exceeds, it limits for growth. Heat stress is considered to be about 10-15˚C increase above the ambient condition and it implies that heat stress in plants ranges between 35-45˚C (Wahid *et al.,* 2007).

If heat stress coincides with critical developmental stages such as flowering, it results in improper pollination and fertilization and ultimately no grain formation (Schoper *et al.,* 1987a). Heat stress in maize results in shortened life cycle (Muchow *et al.,* 1990), reduced light interception (Stone, 2001), increased respiration, reduced photosynthesis (Crafts-Brander and Salvucci, 2002) and pollen sterility (Schoper *et al.,* 1987b). A comparison of the response of male and female reproductive tissues to heat stress demonstrated that female tissues have greater tolerance (Dupis and Dumas, 1990). However, the period between silk and pollination, and ovary fertilization in the female reproductive tissues has also recently been highlighted as a critical period controlling grain yield under heat stress (Cicchino *et al.,* 2010). An increase in temperature from 22 °C to 28 °C during the grain filling period in the US Corn Belt resulted in a 10 % yield loss, while 42 % yield reduction was reported, when mean daily temperatures were increased by 6 °C. (Porter, 2005; Wahid *et al.,* 2007). Under heat stress condition up to 80 per cent reduction in the yield of maize grain was reported by Maukus *et al.,* (2000). To keep pace with climate change effects, especially rising temperature stress, genetic enhancement for heat tolerance in tropical maize has emerged a priority area for maize breeding programme. Maize crop cannot be sown earlier in the month of January, due to lower temperature, which is unfavorable for germination and growth and late sowing is affected by high temperature stress at reproductive stage. Yield is a complex quantitative characters which is affected by a number of its component characters and environment in which plant is grown. The heat tolerance of plant is a complex trait, most probably controlled by multiple genes (Zhag *et al.,* 2005) so the primary aim of maize breeding is to develop heat stress tolerant maize hybrids. Genetic diversity evaluation is frequently used by the breeders as an alternative germplasm selection method. It allows lines to be arranged into groups that, when intercrossed, provide the most promising results which reduce expenses and the time. Identification of heat tolerance inbred lines for development of heat tolerance hybrids for spring season, genetic diversity of inbred lines will play an important role for maize breeding programs. Hence, attempts were made to assess of genetic diversity among inbred lines in normal and heat stress conditions.

Materials and Methods

The experiment was conducted at Maize Section of Bihar Agricultural College of Bihar Agricultural University, Sabour, Bhagalpur, Bihar (India). Twenty nine maize inbred lines were grown for screening heat stress tolerant and susceptible inbred line in normal and heat stress conditions on the two different sowing dates i.e. 30th January 2015, as normal growing condition and 3rd march 2015 as heat stress growing condition, so that in heat stress condition, flowering period of inbred lines may coincide with high temperature (> 32ºC), in Randomized Complete Block Design with three replications. Each plot consisted of 2 rows of 5 meter length with row to row spacing of 60 cm and plant to plant spacing of 20 cm within row. Two seeds per hill were sown by hand dibbling and after one week of germination one seedling from each hill was uprooted to maintain optimum plant population. Recommended agronomic practices were followed to raise the healthy crop. The frequent irrigation was given in heat stress condition to avoid effect of drought stress. Average minimum and maximum air temperature at pollination time was 19.85 ºC and 31.04 ºC in normal condition; and 23.21 ºC and 35.98 ºC in heat stress condition, respectively.

The data were recorded on ten competitive plants, which were randomly taken from each plot in each replication, for the characters, namely, pollen viability, cell membrane thermo stability, days to 50 per cent physiological maturity, plant height, ear height and number of grains per plant. The characters, namely, days to 50 per cent anthesis, days to 50 per cent silk, period shelling per cent and grain yield per plant were recorded on the plot basis. The anthesis-silking interval was calculated as difference between days to 50 per cent silk and days to 50 per cent anthesis. The grain filling period was calculated as difference between days to 50 per cent silk and days to 50 per cent physiological maturity. The 500-seed weight was recorded from the sample of bulk seeds from each plot and each replication. Pollen viability was counted by the method given by Chelong and Sdoodee, 2012 and Cell membrane thermo stability was estimated by the method as given by C.Y. Sullivan (University of Nebraska) in the late 1960's. The mean data of each genotype were subjected to statistical analyses of genetic diversity using Mahalanabis generalized distance (D^2) extended by Rao (1952). Tocher's method was followed to determine the group constellation.

Results and Discussion

Analysis of variance revealed significant differences among the inbred lines for all the thirteen characters studied, this indicated presence of sufficient variability among the maize inbred lines. In normal condition, D^2 statistics displayed that the inbred lines were grouped into six clusters (Table 1a). Cluster IV contained the maximum number of eight inbred lines, indicating overall genetic similarly among them followed by cluster V, VI, I and III having seven, six, four and three inbred lines, respectively while, Cluster II having mono-genotype cluster. Under heat stress condition, the inbred lines were also grouped into six clusters (Table 1b), with cluster I, V and VI containing the maximum of six inbred lines followed by five inbred lines in cluster III and three inbred lines each in cluster II and IV. Clustering pattern of inbred lines under this study reveals that the inbred lines showed considerable genetic diversity among themselves by occupying six different clusters. Similar results were reported by Singh *et al.,* (2005) and Liu Yu Ai *et al.,*

(2006) in maize. In normal condition (Table 2a), the maximum intra-cluster distance was obtained for cluster III (34.23) followed by cluster II (25.91) and cluster I (18.43) values. Furthermore, maximum inter-cluster distance was recorded between cluster II and VI (221.43) followed by cluster IV and VI (145.24), and cluster III and VI (129.82) while, under heat stress condition (Table 2b), the intra- cluster distance was maximum in cluster III (76.03) followed by cluster II (71.35) and cluster I (42.06) indicating wide genetic variability within clusters. The highest inter cluster distance was observed between cluster IV and VI (464.04) followed by cluster III and VI (382.83), cluster V and VI (318.92) which indicated maximum diversity between inbred lines of these clusters. The inter-cluster values were found to be greater in magnitude than intra cluster distance suggesting the presence of diversity among the clusters. It indicated that genotypes included in the same cluster are less divergent than those in different cluster. The high magnitude of D^2 values in different clusters showed that inbred lines were genetically more divergent and may provide basis for consideration in hybridization programme. Therefore, it is suggested that if the diverse inbred lines from these groups along with the desirable heat tolerant traits are used in breeding programmes, it is expected to get better hybrids for high grain yield with heat stress tolerance due to non-allelic interaction. The selected parents for hybridization on the basis of their large inter- cluster distance could be useful for development of heat tolerant maize hybrids. Emphasis should be laid on characters contributing maximum D^2 values for choosing the cluster for the purpose of further selection and choice of parents for hybridization. These findings are in conformity with the findings of Datta and Mukherjee (2004), Singh *et al.,* (2005) and Marker and Krupakar (2009). An attempt was made to characterize the individual inbred lines in respect of their mean values for different characters with a view to getting the idea to be used in breeding programme. In normal condition (Table 3a), cluster VI showed the highest mean values for CMT and the lowest mean value for days to 50% anthesis, days to 50% silk, plant height, ear height and days to 50% physiological maturity, while cluster IV showed the highest mean value for grain yield per plant, grains per plant and grain filling period. Cluster V showed the highest mean value for grain yield per plant and pollen viability. Cluster I and II showed the highest mean value for shelling per cent and 500-seeds weight respectively. Cluster III showed the minimum mean value for ASI. Under heat stress condition (Table 3b), the inbred lines of cluster VI showed the highest mean values for character, grains per plant, grain filling period, CMT and pollen viability, whereas, the minimum mean values for ASI. Cluster IV showed the minimum mean value for days to 50 % anthesis, days to 56 % silk and days to 50% physiological maturity. Cluster II showed the highest mean values for grain yield per plant and shelling per cent. Cluster III showed the minimum mean values for plant height and ear height. Cluster V showed the highest mean values for 500-seeeds weight. The promising inbred lines for grain yield per plant, grains per plant, days to 50% anthesis, days to 50% silk, CMT %, plant height, ear height and days to 50% physiological maturity were identified from cluster IV and VI, on the basis of mean values which could be utilized for hybridization for the development of high yielding maize hybrids under heat stress condition, while in normal condition the promising genotypes for yield and yield attributing traits which had the highest mean value were identified from cluster IV and cluster VI, could be utilized for hybridization programme for the development of high yielding maize hybrids (Khodarahmpour and Choukan, 2011). Based on cluster means Singh and Chaudhari (2003) and Rohman *et al.,* 2015 also reported wide range of variation for grain yield and it's components in maize. Marker and Krupakar (2009) also had assessed the range of variability of 16 genotypes for 14 traits in maize. The present results are in agreement with those of Tang *et al.,* (2002) and Alom *et al.,* (2003) who also identified the above mentioned characters as the principal components contributing maximum to the total variation in maize. Among the characters studied in normal condition (Table 4), maximum contribution was made by plant height (39.90) followed by 500-seeds weight (23.89) and grains per plant (16.01%), while under heat stress condition, maximum contribution was made by 500-seed weight (24.38) followed by plant height (17.98) and shelling % (17.24). The highest contribution towards divergence in this regard was put forth by grain yield per plant similar results were reported by Kumar and Singh (2002), Dutta and Mukharjee (2004), Beyene *et al.,* (2005). Therefore, these characters may be given importance during hybridization programme. The current consequences are in concurrence with those of Tang *et al.,* (2002), Alom *et al.,* (2003), Marker and Krupakar (2009) who also identified above mentioned characters as the principal components contributing maximum to the total variation in maize.

Table.1a Distribution of twenty nine inbred lines of maize under normal condition

Clusters	Number of inbred lines	Name of genotypes included
Cluster I	4	CML27, CML50, CML73, CML305
Cluster II	1	CML130
Cluster III	3	CML33, CML118, CML411
Cluster IV	8	CML19, CML28, CML70, CML139, CML 162, CML304,CML328, CML451
Cluster V	7	CML18, CML25, CML161, CML164, CML165,CML171, CML172
Cluster VI	6	CML474, CML116, CML189, CML308, CML 306, CML307

Table.1b Distribution of twenty nine inbred lines of maize under heat stress condition

Clusters	Number of inbred lines	Name of genotypes included
Cluster I	6	CML27,CML28, CML50, CML73, CML162, CML165
Cluster II	3	CML474, CML411, CML307
Cluster III	5	CML19, CML33, CML116, CML118, CML130
Cluster IV	3	CML304, CML305, CML306
Cluster V	6	CML25, CML70, CML139, CML164, CML171, CML328
Cluster VI	6	CML18, CML161, CML172, CML189, CML308, CML451

Table.2a Average intra (bold values) and inter cluster distance among six clusters of twenty nine inbred lines in maize under heat stress condition

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	**42.05**	127.36	109.92	126.82	122.94	254.04
Cluster II		**71.35**	197.91	249.70	119.24	198.60
Cluster III			**76.02**	145.05	151.37	382.83
Cluster IV				**0.00**	97.58	464.04
Cluster V					**0.00**	318.92
Cluster VI						**0.00**

Table.2b Average intra (bold values) and inter cluster distance among six clusters of twenty nine inbred lines in maize under normal condition

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	**18.42**	54.73	40.00	40.74	27.43	90.32
Cluster II		**25.91**	58.57	44.70	69.06	221.41
Cluster III			**34.23**	68.26	51.26	129.82
Cluster IV				**0.00**	55.63	145.24
Cluster V					**0.00**	90.69
Cluster VI						**0.00**

Table.4 Per cent contribution of characters towards diversity in maize inbred lines under normal and heat stress condition

Sl. No.	Character	Percent contribution	
		Normal condition	Heat stress condition
1.	Days to 50% anthesis	3.20	3.69
2.	Days to 50% silk	0.01	0.01
3.	Pollen viability	0.01	9.11
4.	Anthesis -silking interval	0.01	0.01
5.	CMT percent	1.97	0.49
6.	Plant height	39.90	17.98
7.	Ear height	12.56	2.71
8.	Days to 50% physiological maturity	0.25	0.99
9.	Grain filling period	0.99	11.33
10.	Grains per plant	16.01	11.58
11.	500 -seed weight	23.89	24.38
12.	Shelling percent	1.23	17.24
13.	Grain yield per plant	0.01	0.49

Table.3a Mean values of clusters and contribution of different characters towards genetic divergence of twenty nine inbred lines in maize under normal condition

Clusters	Days to 50% anthesis	Days to 50% silk	Pollen viability	ASI	CMT %	Plant height	Ear height	Days to 50% physiological maturity	Grain filling period	Grains per plant	500-seed weight	Shelling%	Grain yield per plant
1 Cluster	81.89	83.47	96.42	1.61	66.89	103.06	53.28	113.39	31.00	267.00	106.62	82.83	55.44
2 Cluster	82.36	84.44	96.75	2.08	63.56	132.25	65.44	112.33	29.14	235.69	113.68	79.75	52.22
3 Cluster	83.54	85.08	95.83	1.54	64.46	108.42	50.04	112.75	28.54	201.08	91.86	80.67	37.29
4 Cluster	75.33	77.00	92.50	1.67	65.33	132.33	53.33	116.33	41.33	291.67	97.98	82.09	59.00
5 Cluster	83.00	85.00	98.00	2.00	62.67	89.67	35.67	115.33	29.33	273.33	110.53	68.82	59.00
6 Cluster	74.33	76.67	95.33	2.33	67.33	66.00	30.00	109.33	34.33	290.00	76.60	76.38	45.33

Table.3b Mean values of clusters and contribution of different characters towards genetic divergence of twenty nine inbred lines in maize under heat stress condition

Clusters	Days to 50% anthesis	Days to 50% silk	Pollen viability	ASI	CMT %	Plant height	Ear height	Days to 50% physiological maturity	Grain filling period	Grains per plant	500- seed weight	Shelling%	Grain yield per plant
1 Cluster	71.43	75.43	48.52	4.00	53.14	75.05	31.29	95.10	19.67	111.24	52.55	48.66	12.29
2 Cluster	65.67	68.31	54.81	2.67	59.67	80.29	39.38	93.10	24.79	171.05	90.57	64.35	30.95
3 Cluster	68.22	72.17	29.17	3.94	52.06	54.72	21.42	92.92	20.75	117.72	56.71	36.63	13.17
4 Cluster	61.67	67.33	27.67	5.67	41.00	95.67	51.00	88.00	20.67	67.00	41.00	17.33	6.33
5 Cluster	67.33	70.67	42.33	3.33	48.67	92.33	44.33	93.33	22.67	69.67	105.83	30.71	10.67
6 Cluster	69.33	71.50	57.33	2.33	60.33	95.83	44.33	100.17	28.67	250.67	85.00	73.00	40.33

Assessment of genetic diversity is the basic need for the utilization of any inbred line for development of high yielding maize hybrids. The research findings suggested that adequate genetic diversity existed among twenty nine inbred lines. Considering cluster distance, inter-genotypic distance, mean values and other agronomic performances the inbred lines, namely, CML304, CML 305 and CML306 from cluster IV and CML18, CML161, CML172, CML189, CML308, CML451 from cluster VI may be considered as better parents for future hybridization programs to obtain desirable heat tolerant maize hybrids. Similarly, the inbred lines of, cluster IV and cluster VI may be considered as better parents for future hybridization programs to obtain desirable normal maize hybrids in respect of yield and yield contributing. Results indicated that the inbred lines under study are highly diversified under normal and heat stress condition and hence, high heterotic hybrids would be resulted through the crossing of diverse lines clubbed under different clusters as parent in maize breeding programme aimed to enhance grain yield.

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