

## Dissection of heat tolerance mechanism in tropical maize

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### ABSTRACT

Identification of diverse inbred lines and selection of heat tolerant genotypes based on key secondary traits that influence the grain yield is of great importance. In the present study, correlation coefficient and path analysis were used to identify potential traits for heat tolerance. The results on correlation studies revealed that grain yield per plant had positive relationship with number of kernels per cob (0.833), shelling percentage (0.592), chlorophyll content (0.575), plant height, ear height, 100-seed weight, pollen shed duration and cob length. The traits like days to 50% tasseling (-0.383), days to 50% silking (-0.382), anthesis silk interval (-0.381), senescence and cell injury (%) were negatively associated with grain yield. Further, path analysis showed that the number of kernels per cob had highest positive direct (0.6689) and indirect effect via chlorophyll content and days to 50% tasseling on grain yield followed by shelling percentage through number of kernels per cob, days to 50 % tasseling and chlorophyll content. Cluster analysis based on phenotypic data showed presence of three main groups. The first group (G1) consisted of four inbred lines (5.1%) which were superior to mean of 75 inbred lines in respect of all the traits. The selection of genotypes based on number of kernels per cob, anthesis silk interval, shelling percentage, chlorophyll content, plant height and ear height will indirectly increase the maize yield under heat stress.

**Key words :** Heat tolerance, maize, *Zea mays* L.

### INTRODUCTION

Maize (*Zea mays* L. 2n=20) is an important cereal crop next to rice and wheat. In India, maize is predominantly grown as **kharif** crop but some parts of the country follow **rabi** and spring season cropping. Due to its low water requirement, spring cultivation is gaining importance in last few years. Maize cultivation gives 4F (Food, Feed, Fodder and Fuel) advantages to the farmers, providing sustainable income from all its produce. Maize consumption yields 20 to 30% of total calorie intake in human diet in the developing countries like India. The maize is cultivated in

an area of 9.5 mha producing 23.29 mt of grain yield with a productivity of 2450 kg/ha (India Maize Summit, 2014). In country like India where population growth is enormous, there is a demand of 45 million tonnes by 2030 to maintain self-sufficiency and support food security (DMR, 2011).

In recent times, maize production in India is severely hampered by increasing temperatures, especially in **rabi** and spring maize. Heat stress is defined as a transient elevation in temperature, usually 10°C to 15°C above ambient temperature (Wahid *et al.*, 2007). It is predicted that the global mean temperature would rise by 0.3°C per decade

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reaching to approximately 1°C and 3°C above the present value by years 2025 and 2100, respectively (IPCC, 2007). Moreover, a slight increase in temperature by 2°C would reduce maize yield by 13% compared to increased 20% intraseasonal variation which reduces maize yield by only 4.5% (Rowhani *et al.*, 2011; Lobell *et al.*, 2011; Deryng *et al.*, 2014). Very little improvement was done regarding heat tolerant hybrids for tropical and sub-tropical countries (Cairns *et al.*, 2012). To accomplish this task, there is a need to develop climate resilient hybrids that can withstand heat stress. Grain yield, being a complex quantitative trait, depends on number of factors. Therefore, direct selection for yield *per se* may not be the most efficient method for its improvement, but indirect selection for best promising lines based on significantly associated traits would be promising. Improvement of any crop mainly depends on the morphological characterization of genotypes for varied secondary traits that influence the yield levels under specific climatic conditions and later classification of germplasm into distinct groups. The correlation and path analysis are one of the key indices used by many breeders to know the association between the traits and to determine the nature of relationships between dependable variant with its independent variable. Under heat stress, Khodarahmpour (2012) studied correlation among different traits and characterized 28 maize hybrids into four groups by Ward's minimum. In another study, Khodarahmpour and Choukan (2011) reported correlations among traits in maize.

In the present investigation, correlation and path analysis studies were undertaken to provide information on traits that are associated with grain yield and to help in indirect selection of tolerant inbred lines based on associated traits under heat stress. The characterization and clustering of genotypes paves way to identify distinct inbred line useful for heterosis breeding.

## MATERIALS AND METHODS

The present investigation was carried out during summer 2014 at International Maize and Wheat Improvement Center (CIMMYT), Hyderabad, ICRISAT campus, located at 17°53' N latitude and 78°27' E longitude with an altitude of 545 m above mean sea level. The

experimental material consisted of 75 tropical maize inbred lines, which serve as source for developing heat tolerant hybrids. The inbred lines were laid out in a  $\alpha$ -lattice design, randomized and sown in two replications, accommodating 150 plots. Each plot had two rows with net plot size of 3 m length. The inter-row spacing of 75 × 20 cm was maintained. The recommended dose of fertilizers (150 : 75 : 37.5 N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O kg/ha) was applied to the crop.

The following observations were recorded during crop period viz., days to 50% tasseling (AD), days to 50% silking (SD), pollen shed duration (PSD), anthesis silk interval (ASI), plant height (PH), ear height (EH), cob length (CL), cob girth (CG), 100-seed weight (HSW), shelling per cent (SP), number of kernels per cob (NKC), chlorophyll content (CC), senescence (SEN), cell injury % (CI %) and grain yield per plant (GYP). The senescence was scored using a scale from 0 (0% senescence) to 10 (100% senescence) by visual observation. Scale 5 indicated 50% of the plant height from ground level showing symptoms. The cell injury (%) was calculated according to Jamil *et al.* (2012) with some modifications using electric conductivity of leaf samples (Autoranging EC meter, HI 2300).

$$\text{Cell injury (\%)} = \frac{\text{Initial electric conductivity reading (T}_1\text{)}}{\text{Final electric conductivity reading (T}_2\text{)}} \times 100$$

The mean phenotypic data were used for correlation coefficient and path analysis among various traits using TNAU-STAT-statistical package (Manivannan, 2014). Path coefficient analysis was carried out using phenotypic correlation values of yield components on yield as suggested by Wright (1921). The phenotypic diversity among 75 inbred lines was analyzed using MYSTAT Software v12.02 employing Mahalanobis method (MYSTAT Software, Inc., Chicago, IL).

## RESULTS AND DISCUSSION

Selection of high yielding genotypes under heat stress based on correlation and path analysis would supply reliable information on the nature, extent and direction of selection. The results on correlation studies (Table 1) among various traits revealed that grain yield per plant had positive relationship with plant

**Table 1.** Phenotypic correlation coefficients of grain yield and related traits in tropical maize under heat stress

Traits	PH	EH	AD	SD	ASI	PSD	CC	CL	CG	SEN	CI (%)	HSW	NKC	SP
PH	1													
EH	0.863	1												
AD	-0.116	-0.082	1											
SD	-0.035	-0.037	0.905	1										
ASI	0.129	0.075	-0.039	0.374	1									
PSD	0.157	0.085	-0.222	-0.107	0.267	1								
CC	0.209	0.283	-0.207	-0.179	0.026	0.160	1							
CL	0.270	0.314	-0.048	-0.073	-0.063	-0.075	0.140	1						
CG	-0.248	-0.217	0.015	-0.026	-0.116	-0.253	-0.130	-0.065	1					
SEN	-0.286	-0.271	-0.231	-0.224	-0.027	-0.187	-0.209	-0.067	0.352	1				
CI (%)	-0.149	-0.064	0.089	0.061	-0.035	-0.160	-0.220	-0.058	0.749	0.413	1			
HSW	0.421	0.470	-0.218	-0.210	-0.033	-0.018	0.159	0.219	0.016	-0.231	-0.074	1		
NKC	0.199	0.230	-0.449	-0.454	-0.086	0.139	0.431	0.244	-0.052	-0.112	-0.153	0.223	1	
SP	0.101	0.186	-0.368	-0.360	-0.033	0.146	0.379	0.113	-0.214	-0.145	-0.343	0.220	0.717	1
GYP	0.285	0.280	-0.383	-0.382	-0.381	0.213	0.575	0.275	-0.076	-0.267	-0.161	0.277	0.833	0.592

PH : Plant height, EH : Ear height, AD : Days to 50% tasseling, SD : Days to 50% silking, ASI : Anthesis silk interval, PSD : Pollen shed duration, CC : Chlorophyll content, CL : Cob length, CG : Cob girth, SEN : Senescence, CI (%) : Cell injury%, HSW : 100-seed weight, NKC : No. of kernels per cob, SP : Shelling per cent and GYP : Grain yield per plant.

height, ear height, pollen shed duration, chlorophyll content, cob length, 100-seed weight, number of kernels per cob and shelling percentage. Among the positively associated traits, number of kernels per cob (0.8329), shelling percentage (0.5917) and chlorophyll content (0.5753) reported high correlation with grain yield and among themselves. The results were in accordance with Khodarahmpour (2012), who reported positive association between plant height, ear height and number of kernels per cob with grain yield under heat stress in maize hybrids. The strong correlation between ear height and plant height with grain yield suggested that tall plants with high ear placement gave better yield under heat stress. The tall stature of the plant provided space for more number of leaves with good orientation, finally resulting in high photosynthesis rate and dry matter accumulation. It also implied that the tall lines excelled in capacity to support kernel growth through stem reserve mobilization (Al-Tabbal and Al-Fraihat, 2012). Chlorophyll is the basic pigment of plants that leads to accumulation of yield through photosynthesis. In the present study, chlorophyll content showed positive association with grain yield and negative association with senescence owing to increased yield by decreasing the senescence i. e. more green leaves per plant. Under drought stress, the maize cultivars with high chlorophyll content showed more resistance to moisture stress and showed increased yield due to high photosynthates production (Khayatnezhad and

Gholamin, 2012). The yield attributing traits like number of kernels per cob, 100-seed weight and shelling per cent showed positive association with grain yield. In the present investigation, grain yield per plant was negatively associated with days to 50% tasseling (-0.383), days to 50% silking (-0.382), anthesis silk interval (-0.381), senescence (-0.267) and cell injury per cent (-0.161).

The reduced flowering dates and shortened ASI promoted synchronization allow plants to escape heat stress and produce fertile pollen and receptive silk which finally leads to efficient fertilization paving way to more kernel set and higher yield. Magorokosho *et al.* (2003) reported that selection for genotypes with reduced ASI was more effective than grain yield alone under drought stress. Cell injury per cent showed positive correlation with SEN and negative association with PH, EH, CC, NKC, SP and GYP. Increase in cell injury leads to release of reactive oxygen species complexes and leakage of electrolytes which leads to chlorosis, SEN and death of the tissue, leading to inappropriate production of assimilates which are required for proper growth. The cell injury per cent reduces the yield levels indirectly by reducing the contribution of yield attributing traits like PH, EH, CC, NKC and SP. The heat tolerant genotype should have less cell injury per cent (Renu *et al.*, 2004). Plant SEN is a common physiological phenomenon which leads to drying of the leaves. Under stress, the phenomenon proceeds at faster rate moving towards the flag leaf by destroying the

chlorophyll content. Genotypes with slow senescence showed the highest grain yield under drought stress (Guendouz *et al.*, 2012). In the present investigation, SEN was negatively associated with grain yield. Hence, plants that senesce the least are more suitable for cultivation under heat stress. The selection for one of the above traits in direction of correlation would ensure selection for the other traits and result in improved yield (Falconer, 1989).

Path coefficient analysis is used to obtain further information on the interrelationships among traits and their effects on grain yield. The phenotypic direct and indirect effects of yield related traits on grain yield are presented in Table 2. The number of grains per cob showed highest positive direct effect (0.6689) on grain yield followed by days to 50% silking (0.5684), chlorophyll content (0.2572), plant height (0.1661), CI % (0.135), cob length, pollen shed duration and shelling percentage, respectively. Highest negative direct effects were observed for days to 50% tasseling (-0.5725), ASI (-0.2875), ear height (-0.1921), senescence (-0.1687) and cob girth (-0.0381). The NKC via CC and AD had highest positive indirect effect on grain yield followed by SP through NKC (0.4797), AD (0.2106) and CC (0.0974). Cell injury per cent showed positive direct effect on grain yield which is undesirable, this may be due to indirect effect via negative NKC and CC which leads to reduced yield. Moreover, the negative correlation coefficient at phenotypic (-0.1608) level was noticed between cell injury % and grain yield per plant. Senescence had negative

direct effect (-0.1687) on grain yield and indirect effect via most of the traits except ASI and cell injury %. The correlation coefficient also proved negative association with grain yield. Chlorophyll content showed positive direct effect on grain yield with indirect effect via negative cell injury % (-0.13) and positive NKC (0.2882). The selection of genotypes with high chlorophyll content indirectly favours identification of lines with low cell injury %. The pH indirectly affects grain yield through NKC, CC and reduced ASI. The anthesis silk interval showed negative direct effect and proved to be negatively correlated with grain, which shows that decreased ASI resulted in synchronized flowering and good pollination. The negative direct effect of the EH may be nullified by higher magnitude of positive indirect effect of most traits on grain yield. Ear height is considered one of the important characters in maize hybrids. Normally, as the ear height increased, it received more photosynthates from the leaves which ultimately affected the individual grain weight and final yield of the crop.

Clustering analysis based on phenotypic data using Mahalanobis method showed presence of three main groups viz., GI, GII and GIII (Fig. 1). Khodarahmpour (2012) used ward's clustering method to identify the best line suitable for cultivation under high temperatures. To identify the group with promising inbred lines, the overall mean value of 75 inbred lines for individual trait was compared with mean value of respective trait of individual group in the direction of correlation (Table 3). The first group (GI)

**Table 2.** The direct (diagonal, bold) and indirect (out of diagonal) effects of maize yield attributes on grain yield under heat stress

Traits	PH	EH	AD	SD	ASI	PSD	CC	CL	CG	SEN	CI (%)	HSW	NKC	SP	Correlation with grain yield
PH	<b>0.166</b>	-0.166	0.066	-0.020	-0.037	0.010	0.054	0.020	0.009	0.048	-0.020	0.020	0.133	0.000	0.285
EH	0.143	<b>-0.192</b>	0.047	-0.021	-0.022	0.006	0.073	0.024	0.008	0.046	-0.009	0.023	0.154	0.001	0.280
AD	-0.019	0.016	<b>-0.573</b>	0.514	0.011	-0.015	-0.053	-0.004	-0.001	0.039	0.012	-0.010	-0.300	-0.001	-0.383
SD	-0.006	0.007	-0.518	<b>0.568</b>	-0.108	-0.007	-0.046	-0.005	0.001	0.038	0.008	-0.010	-0.304	-0.001	-0.382
ASI	0.021	-0.015	0.022	0.213	<b>-0.288</b>	0.018	0.007	-0.005	0.004	0.005	-0.005	-0.002	-0.058	0.000	-0.081
PSD	0.026	-0.016	0.127	-0.061	-0.077	<b>0.066</b>	0.041	-0.006	0.010	0.032	-0.022	-0.001	0.093	0.001	0.213
CC	0.035	-0.054	0.118	-0.102	-0.007	0.111	<b>0.257</b>	0.011	0.005	0.035	-0.130	0.008	0.288	0.001	0.575
CL	0.045	-0.060	0.028	-0.041	0.018	-0.005	0.036	<b>0.075</b>	0.003	0.011	-0.008	0.011	0.163	0.000	0.275
CG	-0.041	0.042	-0.009	-0.015	0.033	-0.017	-0.033	-0.005	<b>-0.038</b>	-0.059	0.101	0.001	-0.035	-0.001	-0.076
SEN	-0.048	0.052	0.132	-0.128	0.008	-0.012	-0.054	-0.005	-0.013	<b>-0.169</b>	0.056	-0.011	-0.075	-0.001	-0.267
CI (%)	-0.025	0.012	-0.051	0.035	0.010	-0.011	-0.057	-0.004	-0.029	-0.070	<b>0.135</b>	-0.004	-0.103	-0.001	-0.161
HSW	0.070	-0.090	0.125	-0.119	0.010	-0.001	0.041	0.016	-0.001	0.039	-0.010	<b>0.048</b>	0.150	0.001	0.277
NKC	0.033	-0.044	0.257	-0.258	0.025	0.009	0.111	0.018	0.002	0.019	-0.021	0.011	<b>0.669</b>	0.002	0.833
SP	0.017	-0.036	0.211	-0.205	0.010	0.010	0.097	0.009	0.008	0.024	-0.046	0.011	0.480	<b>0.003</b>	0.592

Trait details are given in Table 1.

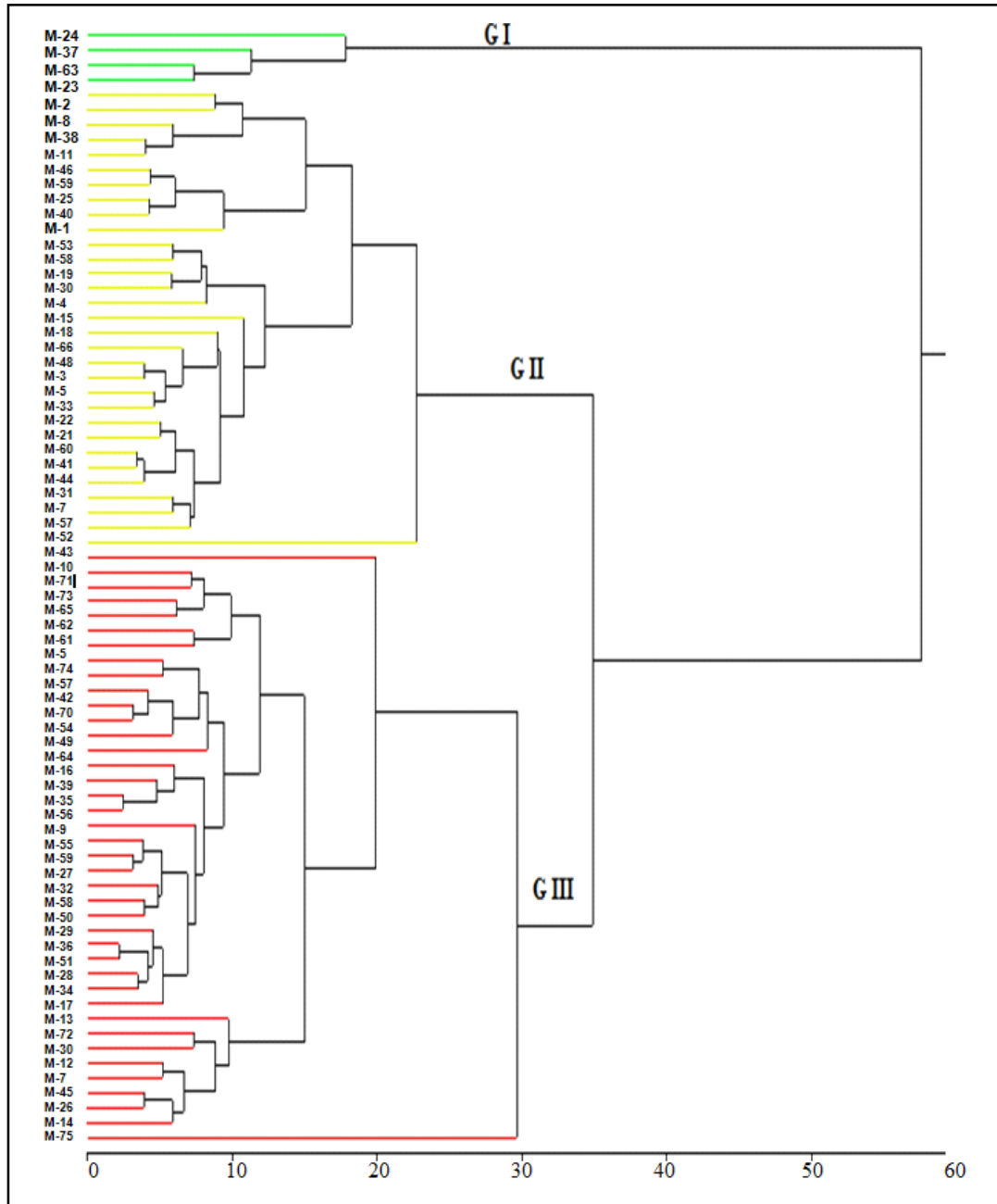


Fig. 1. Dendrogram showing clustering of 75 inbred lines into three groups.

Table 3. Comparison of total mean value of each trait with respective group mean value

Traits	PH	EH	AD	SD	ASI	PSD	CC	CL	CG	SEN	CI (%)	HSW	NKC	SP	GYP
Over mean	99.41	55.69	57.87	59.68	1.92	3.42	17.17	9.15	9.93	5.53	15.90	16.26	67.41	51.61	4.83
GI mean	118.83	67.20	52.53	54.57	2.07	4.72	26.64	10.64	10.03	4.50	14.76	20.47	243.58	77.82	25.86
GII mean	100.21	56.52	58.33	59.80	1.65	3.26	16.54	9.37	8.39	4.93	13.64	15.60	85.26	61.01	5.64
GIII mean	96.93	53.96	58.04	60.09	2.10	3.42	16.72	8.85	11.04	6.07	17.67	16.34	37.16	42.18	2.18

Trait details are given in Table 1.

consisted of four inbred lines (5.1 %) which were superior to overall mean of 75 inbred lines in respect of all the traits, namely, high grain yield per plant, chlorophyll content, NKC and

reduced ASI, senescence and cell injury per cent. This group combines both early maturing (drought escape) and drought tolerant genes (short ASI) in the same genotype in addition to

favourable alleles for most of the traits. This group represents promising lines with favourable alleles, which can be used for breeding heat tolerant maize. The second group (GII) consisted of 30 inbred lines (40%) with trait mean value on par with overall mean value for most of the traits except NKC, SP and GYP which showed higher than overall mean value. Under this group, the following inbreds viz., M2, M11, M46 and M53 were proved to be the best and had positive yield attributing traits. Likewise, the genotypes in group three (GIII) (54.6%) reported low mean value for all the traits compared to total mean value, revealing their sensitive nature to high temperature. This group of lines can be improved by population improvement.

In conclusion, the selection of genotypes based on number of kernels per cob, ASI, shelling percentage, chlorophyll content, plant height and ear height will indirectly increase the maize yield under extreme high temperatures. The clustering of genotypes into three distinct groups based on phenotypic data showed the presence of three groups and led to identification of superior inbreds lines.

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