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Deciphering variability for heat tolerance index in maize hybrids and identification of stress resilient heterotic combinations

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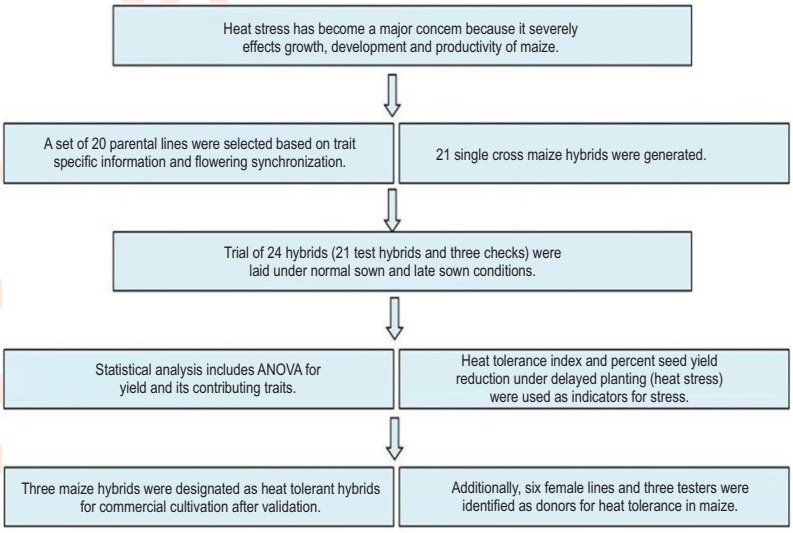
Abstract

Aim: High temperature at any critical stage for a prolonged duration is documented to negatively affect the growth and yield related processes in maize. Therefore, the present study was planned to identify resilience of different heterotic combinations to heat stress under field conditions.

Methodology: A set of 20 parental lines were used as parents and 21 single cross hybrids raised in kharif 2020 using 16 lines as female parent and four lines as male parent along with three commercial checks using randomized block design under two regimes: Normal Sownin mid of February, *i.e.,* timely sown condition (spring season crop) and late sown in mid of March (heat stress crop). Data was recorded for yield and its contributing traits.



Results: Heat stress led to early onset of flowering, thus resulting in shortening of vegetative phase. Consequently, days to grain filling period was found to be reduced (84-106 days) under late sown as compared to 100-123 days under normal sown condition. Amongst the top performing six hybrids in both normal sown and late sown conditions, three hybrids were common *viz.,* JH 20003, JH 20015 and JH 20018, though with different ranking, inferring their wider adaptability and tolerance to heat stress. Under late sown conditions, days to grain filling (r^2=0.55) and thousand seed weight (r^2 =0.50) showed significantly positive correlation with grain yield while as highly significant and negative association was observed between anthesis silking interval and grain yield (r^2 = -0.76). Among the 21 hybrids, three hybrids (JH 20001, JH 20004, and JH 20015) showed greater potential under heat stress conditions on the basis of yield performance under stress, percent yield reduction and heat tolerance index.

Interpretation: Based on multiple selection criteria, three hybrids *viz.,* JH 20001, JH 20004, and JH 20015 were suggested for commercial utilization after validation. This study also led to identification of donor inbreds which can contribute to breeding for heat stress tolerance.

Key words: Grain yield, Heat tolerance index, Heat stress, Maize, Percent yield reduction

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Introduction

Maize is the most versatile food crop grown on 193 m ha area in more than 170 countries across the globe with annual production of 1147 million tonnesand productivity of 5.82 t ha^{-1} (Erenstein *et al*. 2022). As C_4 crop, it is relatively more climate-resilient and can be grown in tropical, sub-tropical and temperate climates from sea level to 3000 m above sea level. In India, it is cultivated over an area of 9.0 million hectares with a production of 28.86 million tonnes and a productivity of 3.1 tonnes ha^{-1} (Yousuf *et al*. 2021). Under rice-wheat status quo of the Indian food system and need for alternate pathways toward healthier food baskets with less environmental burden, maize has emerged as a suitable candidate for crop diversification in North-western plains of India (Parihar *et al.,* 2018). Moreover, the diversified uses of maize in feed, food, industry and bioethanol ensures the sustainable demand of crop.

The recent advances in the production of silage bales and the synthesis of fuel ethanol from maize have opened up new vistas towards large scale maize cultivation in the region. In this region, the farmers are growing maize in two seasons- spring season from end January to May and kharif season from June to October. The productivity is higher in spring season due to long crop duration, less weeds and insect-disease pressure, however, the potential productivity is affected by prolonged high temperature regimes (or heat stress) during most of the critical crop growth period, starting from the late vegetative stage until early grain filling phase (Prasanna, 2011). Moreover, farmers generally go for delayed sowing in March. Heat stress at the time of crop reproductive stage is more detrimental to productivity in comparison to vegetative stages and can cause entire loss of grain cycle (Lobell *et al.,* 2011). It ultimately leads to reduction in grain filling duration period, thereby affecting the crop productivity (Zhang *et al.,* 2006).

The optimum temperature for maize plant in day and night times ranges from 22 to 32°C and 16.7 to 23.3°C, respectively, where the photosynthetic rate is observed to be higher than respiration resulting in increased plant growth (Naveed *et al.,* 2014). Temperatures above 35°C for a prolonged period is unfavourable for crop growth and above 40°C, particularly during flowering and grain filling stages, may lead to severe yield losses (Tesfaye *et al.,* 2017) due to decreased photosynthetic rate, silk desiccation and pollen viability (Noor *et al.,* 2019). Izaurralde *et al.* (2011) reported that each degree rise in mean seasonal temperature would reduce the economic yield of maize crop by 3–13%. Heat stress tolerance in maize remains a crucial concern to physiologists and plant breeders for sustainable productivity as nature and intensity of damage to crop varies extensively due to complexity of the trait.

Other than morphological changes, several physiological and biochemical changes (photosynthetic acclimation, stalk sugar mobilization, chlorophyll content), and reproductive organ malfunctioning (low pollen viability, silkdesiccation, lack of fertilization, embryo abortion, shrunken kernels) are known to be associated with heat stress in maize (Wang *et al.,* 2021). Prolonged anthesis-silking interval, reduction in kernel set, decreased photosynthesis rate and damaged cellular membrane have also been reported under heat stress conditions (Longmei *et al.,* 2021).

Breeding for heat stress tolerance in maize is primarily aimed at identifying genotypes with optimal reproductive capacity and low yield loss under heat-stress conditions as compared to non-stress conditions. Selection based on grain yield alone under heat stress is often misleading due to its low heritability (Edmeades *et al.,* 1993). Therefore, it is very essential to spot fewer complex traits related to heat stress which are highly associated with grain yield and have relatively high heritability (Betran *et al.,* 2003). Therefore, the present study was planned to identify the key secondary traits associated with heat stress tolerance in 21 single cross hybrids of maize that will help the plant to maintain productivity under heat stress environment. Further, the study also enabled to identify parental lines which can be utilised as donors to breed heat stress resilient and highly yielding cultivars.

Materials and Methods

The present study was conducted during *kharif* 2020 to spring 2021 at research farms of Punjab Agricultural University, Ludhiana. A set of 20 parental lines consisting of inbreds derived from different heterotic pools; semi exotic and indigenous lines; lines collected from different institutes *viz.,* CIMMYT Asia Hyderabad, IIMR and NBPGR, New Delhi. Out of these 20 parental lines, 16 inbred lines were selected as female lines and four inbred lines as male parent were selected based on reproductive synchronization and pre-determined heterotic grouping and used to raise 21 single cross hybrids through hand pollination in kharif 2020. In spring 2021 F_1 seed of 21 crosses along with three commercial checks *viz.,* PMH 10, P1844 and DKC9108 were evaluated under two regimes: (I) normal sown in mid of February (during recommended sowing time for spring season crop) and (ii) late sown in mid of March to expose the crop to heat stress during flowering and grain filling stage.

Under each condition, a trial of 24 hybrids (21 test hybrids +3 checks) was conducted in a randomized block design with two replications and row to row and plant to plant spacing of 60 cm and 20 cm, respectively. The crop was raised using recommended practices to full maturity and evaluated for yield and its contributing traits. Observations on various traits *viz.* plant height (cm), ear height (cm), ear: plant height ratio, 1000 seed weight (g) and grain yield per hectare (kg) were recorded on five randomly selected competitive plants, except for maturity traits *viz.,* days to 50% anthesis, days to 50% silking, anthesis-silking interval, days to 75% brown husk and days to grain filling where data were recorded on plot basis. Heat tolerance index was determined by the formula given by Fernandez (1992).Statistical analysis of phenotypic data was conducted using SAS software (SAS Institute, 2011).

Results and Discussion

The maximum mean temperature recorded for late sown condition was>35º C from late vegetative stage until grain filling stage which was above the optimum range for normal growth and development of the crop and satisfies the requirement for evaluation under heat stress condition. The critical growth stages in maize in concordance with maximum temperature and minimum temperature under normal and late sown conditions (Fig. 1). It was found that heat stress resulted in shortening of vegetative phase and consequently led to early onset of flowering. The period for days to grain filling was found to be reduced (84 to 106 days) under late sown condition as compared to 100-123 days under normal sown regime. The results revealed that the heat stress caused considerable changes in the agro-morphological and grain yield traits in the test hybrids evaluated under study (Table 1, 2). In normal sown conditions, the mean plant height was 235 cm and mean ear height was 139 with mean ear to plant height ratio of 0.50, whereas under late sown conditions, the mean values for plant height, ear height and ear to plant height ratio was found to decrease.

The average value for anthesis-silking interval was found to be 2, indicating well synchronization among female and male lines to generate hybrids. The mean value for grain yield was 8559 kg ha^{-1} with mean 1000 seed weight of 300 g. The comparison of mean values for yield contributing traits inferred that plant height, ear height and resulting ear to plant height ratio decreased under heat stress. The results were in agreement with the previous finding as reported by Hussain *et al.* (2019) where heat stress in combination with drought stress significantly decreased plant height in two maize hybrids. Rahman *et al.* (2013) and Longmei *et al.* (2021) also observed similar results with respect to plant height and ear height in double haploid lines of maize and plant height in 25 maize hybrids, respectively, evaluated under heat stress. It was observed that the mean anthesis-silking interval showed three folds increase under heat stress in comparison to normal conditions. Similar results for prolonged anthesis-silking interval under heat stress were found by Longmei *et al.* (2021) and Alam *et al.* (2017). The reason behind the fact is that heat stress leads to reduced time for taselling due to accelerated developmental process and simultaneously delay in silk emergence is observed as a result of decreased partitioning of assimilates to the developing ear.

Delayed silking has been associated with barrenness (Herrero and Johnson, 1980), and appears to reflect reduced partitioning of assimilates to the developing ear at flowering stage (Edmeades *et al.,* 1993). The association of shorter anthesis silking interval has been well documented previously to be associated with higher number of ears per plant (Bolanos and Edmeades, 1993; Edmeades *et al.,* 2000) and also with grain yield. Therefore, anthesis-silking intervall was proposed to be an important trait to improve yield under stress environments (Bolanos 1995; Edmeades *et al.,* 1999). Under heat stress condition in the present study, the maturity has been fastened as

Fig. 1: Reproductive phase comparison of maize test hybrids for normal sown and late sown conditions in relation to maximum temperature.

Table 1: Field performance of maize hybrids for yield and its contributing traits under optimal conditions

Hybrid	Plant height (cm)	Ear height (cm)	Ear: Plant height ratio	Anthesis to silking interval (days)	Days to 75% brown husk	Days to grain filling	1000 seed weight (g)	Grain yield (kg ha^{-1})
JH 20001	205	109	0.53	2	105	33	274	8765
JH 20002	225	130	0.58	2	108	36	354	8664
JH 20003	240	153	0.64	2	111	39	314	9686†
JH 20004	218	145	0.67	1	109	37	284	8620
JH 20005	250	148	0.59	2	108	33	313	8902
JH 20006	230	137	0.60	1	107	37	311	8511
JH 20007	248	142	0.57	2	116	34	286	8376
JH 20008	232	130	0.56	2	107	28	234	6739
JH 20009	243	138	0.57	1	114	37	362	9877†
JH 20010	242	138	0.57	2	103	28	305	8799
JH 20011	228	135	0.59	2	103	28	238	7306
JH 20012	242	158	0.65	3	119	44	343	8908
JH 20013	248	158	0.64	2	117	43	284	9601†
JH 20014	260	168	0.64	2	112	42	266	9303†
JH 20015	250	137	0.55	2	112	41	306	9379†
JH 20016	193	132	0.69	2	106	32	288	6791
JH 20017	248	155	0.63	2	108	33	269	8730
JH 20018	243	143	0.59	3	102	39	356	9283†
JH 20019	252	147	0.58	2	105	31	291	7657
JH 20020	240	137	0.57	3	105	27	332	7917
JH 20021	190	108	0.57	2	108	34	298	6195
PMH 10	240	127	0.53	1	104	31	294	8277
DKC 9108	240	148	0.61	2	112	35	289	8735
P 1844	242	125	0.52	1	118	37	301	9091
Mean	235	139	0.59	2	109	35	300	8504
CD (5%)	21.3	10.8	-	0.54	5.6	4.8	25.3	952
CV %	10.5	7.1	-	0.03	6.98	9.65	5.78	11.4

†Indicates numerically higher value than the best check

indicated by lower mean values for days to 75% brown huskand days to grain filling as compared to normal sown conditions. Therefore, the present study confirmes that heat stress resulted in shortening plant maturation thereby, causing reduction in grain growth duration (Rahman *et al.,* 2005) which ultimately produce smaller grains. The results are in uniformity with the results of Rahman *et al.* (2013). A considerable reduction in the mean value for 1000 seed weight in the test hybrids was revealed under heat stress condition (260 g) in relation to normal sown condition (300 g). Similar response under heat stress was reported by Hussain *et al.* (2019). The average yield of the test hybrids was 8558 kg ha^{-1} under normal sown conditions which declined to 7124 kg ha^{-1} in late sown condition. Khodarahmpour *et al.* (2011) found reduction of grain yield up to 70% in maize inbred lines in high temperature environment. Lower grain yield was associated with adverse effects of high temperature on pollen viability and fertilization (Rowhani *et al.,* 2011). Furthermore, under stressful environmental condition, genetic improvement can be achieved by selecting of secondary traits having high heritability and significant association with grain yield (Betran *et al.,* 2003).

A positive significant correlation was observed between grain yield with days to grain filling (r^2 =0.65), days to 75 % husking (r^2 =0.50) and thousand seed weight (r^2 =0.42) under normal sown conditions. Plant height (r^2=0.05), ear height (r2=-0.10), ear to plant height ratio (r^2=-0.13) and anthesis-silking interval (r^2=-0.13) showed non-significant correlation with yield under normal sown conditions. Under late sown conditions, days to grain filling (r^2=0.55) and thousand seed weight (r^2=0.50) showed significantly positive correlation with grain yield while as highly significant and negative association was observed between anthesis-silking interval and grain yield (r^2= -0.76) which could be attributed to the fact that stress in pre-anthesis stage results in wider anthesis-silking interval leading to barrenness in plants, while absorption of fertilized structure and reduced ear growth rate lead to reduction in kernel number, ultimately affecting crop yield (Cicchino *et al.,* 2010). Chapman and Edmeades (1999) have reported that anthesis-silking interval is highly correlated to grain yield under drought stress and similar trend was reported in our study under heat stress.

The traits were found to be strongly associated with the reproductive potential of crop that could be used as potential surrogates to supplement productivity under heat stress. Plant height (r^2=0.21), ear height (r^2=0.04), ear to plant height ratio (r^2=-

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Table 2: Field performance of maize hybrids for yield and its contributing traits under heat stress conditions

Hybrid	Plant height (cm)	Ear height (cm)	Ear: Plant height ratio	Anthesis to silking interval (days)	Days to 75% brown husk	Days to grain filling	1000 Seed weight (g)	Grain yield (kg ha^{-1})	% yield reduction
JH 20001	215	108	0.50	3	101	37	254	8692†	0.83
JH 20002	223	118	0.53	4	90	24	228	6978	19.46
JH 20003	232	137	0.59	2	97	31	289	7890	18.54
JH 20004	205	120	0.59	2	102	35	280	8529†	1.06
JH 20005	233	120	0.52	3	97	30	308	7603	14.59
JH 20006	228	139	0.61	3	96	33	233	7256	14.75
JH 20007	238	142	0.60	3	105	33	260	7427	11.33
JH 20008	228	130	0.57	5	94	27	204	5254	22.04
JH 20009	235	108	0.46	3	89	32	247	7385	25.23
JH 20010	228	120	0.53	3	100	31	261	6742	23.38
JH 20011	250	105	0.42	7	95	27	240	5864	19.74
JH 20012	Yd	135	0.61	4	100	31	249	6703	24.75
JH 20013	225	118	0.52	3	99	37	254	6884	28.30
JH 20014	248	130	0.53	5	105	39	244	6349	31.75
JH 20015	223	123	0.55	2	107	36	287	8500†	9.37
JH 20016	210	140	0.67	5	95	24	253	5960	12.24
JH 20017	235	125	0.53	6	98	27	234	6408	26.60
JH 20018	220	113	0.51	3	102	31	313	7916	14.73
JH 20019	243	128	0.52	3	104	28	239	6786	11.38
JH 20020	205	115	0.56	3	105	35	261	6759	14.63
JH 20021	230	113	0.49	7	96	25	297	5403	12.78
PMH 10	220	120	0.55	4	91	27	266	7075	14.52
DKC 9108	233	100	0.43	4	103	31	266	8063	7.69
P 1844	228	125	0.55	3	98	33	278	8401	7.59
Mean	227	122	0.54	3.75	99	31	260	7117	16.31
CD (5%)	16.4	11.4	-	1.2	7.3	5.9	21.3	854	
CV %	9.3	7.6	-	0.96	8.89	10.2	7.89	13.6	

†Indicates numerically higher value than the best check

0.16) and days to 75% husk (r^2=0.36) showed non-significant correlation with yield under late sown conditions. The private sector hybrid P1844 was recorded as the best check in the present study under both regimes and six PAU bred advance hybrids *viz.,* JH20009, JH20003, JH 20013, JH 20015,JH 20014 and JH 20018 yielded statistically at par to P1844 under normal sown condition. The results further revealed that only three hybrids *viz.,* JH20001, JH 20004 and JH20015 performed numerically higher than P1844 under heat stress condition.

The hybrids entirely differed in their performance in normal and late sown conditions. The top performing hybrid JH 20001 which ranked first in yield performance in late sown condition was at rank 12 in normal sown regime. The hybrid JH20004 which was at rank three in late sown condition was at rank 15 in normal sown condition. The differential behaviour of hybrids depicted that it is imperative to assess the performance of hybrids under stress conditions for commercial recommendation. Furthermore, amongst the top performing six hybrids in both normal and late conditions, three hybrids were common *viz.,* JH 20003, JH 20015 and JH 20018, though with different ranking, inferring their wider adaptability and tolerance to heat stress. The percent yield reduction ranged from 0.83 to 31.75, indicating wide variability in test hybrids and hence, good scope for selection (Table 2). Further, rigorous sieving based on percent yield reduction, it was found that out of six top performing hybrids under heat stress, three hybrids *viz.,* JH 20001 (0.83); JH 20004 (1.06) and Jh20015 (9.37) were of high resilience. JH 20001 was developed from exotic line ECEC639211-2 obtained from National Bureau of Plant Genetic Resources, New Delhi; JH 20004 and JH 20015 was developed from lines obtained from CIMMYT, Hyderabad. The observations also indicated the utility of wider genetic resources to breed for abiotic stress tolerance. This also infers to prefer multiple criteria to assess heat tolerance in genotypes. LM 21, male parent of five top performing hybrids under heat stress, was identified as donor parent which can contribute to breed for high temperature resilient germplasm.

The heat tolerance index, a reliable indicator to determine trait performance of hybrids under temperature stress ranged from 0.46 to 1.16, depicting differential behaviour of hybrids to heat stress. Kandel *et al.* (2019) and Khodarahmpour *et al.* (2021) determined different stress tolerance indices and also reported that these indices were more accurate criteria for selecting heat

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tolerant and high yielding lines. Hybrids JH 20001, JH 20004 and 20015 exhibited better yield under stress conditions (8692, 8529 and 8500 kg ha^{-1}) and high heat tolerance index (1.04, 1.0 and 1.09). These hybrids with high yield performance, good agronomic traits, percent yield reduction<10 and heat tolerance index>1.0 were designated as hybrids with high heat tolerance and suggested for commercialization after multi-site performance validation and stability. Heat stress causes reduction of plant growth duration due to increased growth rate and ultimate shorter life cycle of crop. Temperature >1-2°C than the normal leads to reduction in grain filling duration and negatively affects yield and yield attributing traits (Zhang *et al.,* 2006; Lobell *et al.,* 2011). Heat stress conditions leads to impaired cell division in both male and female organs, pollen tube germination and growth, ovule viability, anomaly in position of stigmatic and style, number of pollen per silk during fertilization, poor growth endosperm, pro-embryo and barren embryo. These mechanisms are also responsible for production of sterile plants (Yun-Ying *et al.,* 2008).

Overall, the experimental results of the present study led to identification of donor lies and resulting hybrids harbouring tolerance to heat stress, agronomic traits for consideration of selection for heat tolerance and heat tolerance index as one of the important criteria to breed for heat stress tolerance in maize.

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