

CHARACTER ASSOCIATION STUDY IN MAIZE HYBRIDS DEVELOPED THROUGH INTEGRATION OF RAPID CYCLE GENOMIC SELECTION AND DOUBLED HAPLOID TECHNOLOGY FOR HEAT STRESS TOLERANCE

Abstract

Heat stress is becoming a major constraint for maize production; therefore heat stress resilience has emerged as an important aspect in maize hybrids targeted for post rainy spring season. Selection of genotypes based on high grain yield under heat stress condition is often misleading and the identification of secondary traits also associated with grain yield may help in development of heat tolerant cultivars. Hence, the present research work to study the association of traits was conducted during summer and *kharif* 2018 and *rabi* 2018-19 at Bheemaranagudi and Raichur using 111 testcross progenies of doubled haploids derived from C₁, C₂ and C₃ cycles of multi-parental synthetic population 1 and 2 improved through rapid cycle genomic selection for heat stress tolerance. The phenotypic correlation analysis under heat stress and optimal conditions, number of kernels per cob and cob girth exhibited the strong positive association with grain yield per plant. Further, under early spring condition number of kernels per cob, cob length, cob girth and 100 grain weight recorded the strong positive association with grain yield per plant. However, when considering across heat stress, early spring and optimal conditions, the grain yield per plant showed positive and significant strong to moderate association with the traits *viz.*, number of kernels per cob, cob length and cob girth under all the seasons. In addition, days to 50% silking showed significant negative correlation with grain yield per plant under heat stress and optimal condition. Hence, the simultaneous selection criteria for the genotypes that exerts early silking, higher number of kernels per cob, higher cob girth and lengthy cob should be the priority of breeders to achieve higher grain yields in maize under heat stress condition as well as optimal conditions.

Keywords: Maize, heat stress, multi-parental synthetics, rapid cycle genomic selection, doubled haploid, phenotypic correlation

Introduction

The impact of climate change is of global concern. Climate change will be the major impediment to agriculture and it will be greatest in the tropics and subtropics. In relation to this, South Asia is likely to be more vulnerable to multiple stresses and low adaptive capacity [1]. Climate change will have direct or indirect cascading effects on agro-ecosystem, agricultural production and other livelihoods associated with agriculture. These effects profoundly translate into economic and social consequences; ultimately affecting food security in millions of resource-constrained smallholders [2]. The rapidly ever increasing

human population is an alarming issue and the current food production rate combined with population growth and predicted impacts of climate change will not suffice to meet future food demand and this would need more food production under changing climate. Improving crop productivity and livelihoods of smallholders under increasing climate variability will require a multi-disciplinary approach towards crop genetic improvement [3]. In this relation, maize plays a critical role in ensuring food and nutritional security and livelihoods of millions of resource-constrained smallholders in the world's poorest regions in sub-Saharan Africa (SSA), Asia and Latin America, where in maize is predominantly grown under rainfed conditions, but these regions also often under the threat of devastating diseases and insect pests[4].

Maize is highly productive under optimal environmental and crop management conditions. In recent years, among many abiotic stresses, heat stress and temperature fluctuation are becoming key issues to be addressed for upraise in maize production. Maize crop can survive brief exposure to high temperature. But, exposure to temperatures above 35°C for a prolonged period is considered unfavourable for crop growth and beyond 40°C, particularly during flowering and grain filling stage can have severe impact on grain yields [5]. Grain yield losses in maize from heat stress are expected to increase owing to a higher temperature during the growing season. This situation demands the development of maize hybrids tolerant to heat stress without compromising grain yield and quality under stress conditions [6, 7]. Hence, heat stress resilience has emerged as an important trait in maize hybrids targeted for post-monsoon spring cultivation in large parts of South Asia and many other parts of the tropics.

The adverse effects of heat stress can be mitigated by developing crop plants with improved thermo tolerance using various genetic approaches. Genomic Selection (GS) has been a very effective strategy in maize breeding. It exploits high density marker information to predict the total genetic value (Genomic Estimated Breeding Value, GEBV) of an individual based on a model set of training individuals that are genotyped and phenotyped at representative locations. Rapid Cycle Genomic Selection (RCGS) procedure involves integration of GS in breeding programme, which reduces length of the breeding cycle (selection plus recombination of favourable alleles) to a single season. In subsequent generations, intermated individuals are only genotyped and their genetic values are predicted based on previously estimated marker effects. Recent studies in maize for RCGS have reported that, GEBV enabled selection of superior phenotypes resulted in rapid genetic gain for drought and heat tolerance [8, 9, 10, 11, 12]. Cost and time-effective development of homozygous lines is an important component in maize breeding. Doubled Haploid (DH) technology has become an integral part of many commercial maize breeding programmes as DH lines offer several economic, logistic and genetic benefits over conventional inbred lines. The induction and subsequent doubling of maternal haploids is an efficient alternative to generate homozygous lines in quick timeframe (two generations), which otherwise takes longer time in the conventional inbreeding procedure. Further, new advances in DH technology continue to improve the efficiency of DH line development and fuel its increased adoption in breeding programmes worldwide [11, 12, 13].

The prime objective of crop breeding programme is always aimed at increasing the yield of any crop that requires contribution not only of yield but also of its associated traits that could have direct or indirect impact on yield. Polygenes are highly sensitive to the environment. Yield is a polygenically inherited character and hence, the selection of superior genotype based on yield alone may not be effective. Although variability estimates provide information on the extent of improvement, they do not show the extent and nature of relationship which exists between the characters. This could be obtained from simple association analysis. Knowledge of the association of component characters with single plant yield may greatly help in making selection precise and accurate. Breeding for heat stress tolerance in maize had limited success, primarily because selection in stress tolerance breeding programs is often based on grain yield *per se*, which could be misleading in stressed trials owing to its low heritability estimates. Past studies have suggested the use of secondary traits along with grain yield in obtaining realistic genetic gains in breeding for tolerance to abiotic stresses [14]. The approach of using secondary traits to complement phenotypic selection based on yield can improve selection response to a large extent, as heritability of some secondary traits remains high under stressed environment and is often genetically correlated with yield under stress. Thus, breeding for stress tolerance can be complemented by identifying key secondary traits that are closely associated with yield under stressed environments [15].

Most of the heat stress research has been conducted on temperate maize germplasm for high production. However, systematic breeding efforts for improving the tropical maize cultivars with high temperature tolerance have recently been initiated with “Heat stress resilient maize for South Asia (HTMA)” programme through a public-private partnership at CIMMYT (Asia), ICRISAT, Hyderabad, India. Under collaborative research, many researchers have identified several morpho-physiological and yield attributing traits *viz.*, ears per plant, kernel number per cob, plant height, cob height, cob length and girth, chlorophyll content, leaf firing, tassel blast and anthesis-silking interval etc. which are associated with higher grain yield under heat stress tolerance in maize [16, 17, 18, 19, 14, 20, 21]. In addition to these, in the present study, we are reporting the association of morpho-physiological and yield attributing traits with the grain yield by evaluating the DH testcross hybrids involving DH lines from improved cycles of MPS 1 and MPS 2 populations through the integration of genomic selection and doubled haploid technology under heat stress, optimal and early spring conditions.

Materials and methods

Experimental location

The present experiment was laid out (Table 1) at Agriculture College Farm, Bheemaranagudi situated at 16° 72' N Latitude, 76° 80' E Longitude with an altitude of 458 m above mean sea level (MSL) and at Main Agricultural Research Station Farm, Raichur situated at 16° 19' N Latitude, 77° 31' E Longitude with an altitude of 407 m above MSL. Bheemaranagudi and Raichur come under north-eastern dry zone of Karnataka, India. The weather data during crop growth period indicated that the most of the cropping period during summer 2018 was under heat stress. Thus, the combination of high temperature ($T_{max} > 35^{\circ}\text{C}$

Table 1. Details of locations and environments used for evaluation of maize testcrosses involving DH lines from C₁, C₂ and C₃ of MPS 1 and MPS 2 populations and checks during 2018-19

Location	Latitude	Longitude	Season and Year	Environment	Description of the environment	Mean Temperature (°C)		Total rainfall (mm)	Mean RH (%)
						Min.	Max.		
Bheemarayanagudi, Karnataka	16° 72' N	76° 80' E	Summer 2018 (March - June)	Heat stress	Natural heat stress under irrigation	25.10	40.33	4.10	40.98
			<i>Kharif</i> 2018 (August - November)	Optimal	Rain-fed with protective irrigation	20.60	33.33	118.60	66.23
			Late <i>rabi</i> 2019 (January - April)	Early spring	Natural heat stress at post flowering stage of crop growth under irrigation	19.79	36.45	0.04	46.23
Raichur, Karnataka	16° 19' N	77° 31' E	Summer 2018 (March - June)	Heat stress	Natural heat stress under irrigation	25.37	37.81	27.40	48.00
			<i>Kharif</i> 2018 (August - November)	Optimal	Rain-fed with protective irrigation	22.10	33.05	160.40	61.00
			Late <i>rabi</i> 2019 (January - April)	Early spring	Natural heat stress at post flowering stage of crop growth under irrigation	21.03	34.63	23.50	48.00

and $T_{\min.} \geq 21$ °C) and relative humidity (< 50%) *i.e.*, the warm and dry humid climatic conditions of the locations were appropriate and ensured proper evaluation of maize DH testcrosses under high temperature regime for heat stress tolerance.

Experimental material

The experimental material comprised of DH derived from multi-parental synthetic populations (MPS) namely MPS 1 and MPS 2, that were constituted for heat stress tolerance by CIMMYT-Asia Regional Programme, ICRISAT, Hyderabad using 8 to 10 heat tolerant elite Asia adapted lines belonging to heterotic group A (HGA) and heterotic group B (HGB), respectively. To constitute the multi-parental synthetics, the elite heat resilient parental lines from each population were intermated in half-diallel design to obtain the F_1 progenies. The F_1 progenies were intermated and approximately 500 S_2 families from each population were derived through selfing the intermated bulks. The selfed S_2 families were testcrossed with tester line from the opposite heterotic groups and the testcross progeny were evaluated (phenotyping) under managed heat stress and well-watered conditions for various traits. In addition, each of the S_2 families were subjected to genotyping with polymorphic SNP markers for use in prediction models for grain yield estimation under heat stress. A selection intensity of 10% was used to intermate the S_2 families. The balance bulk from these intermated crosses formed the cycle 1 (C_1).

The C_1 seeds from each MPS population were planted in nearly 50 rows and leaf samples were collected from every plant for DNA extraction. Genotyping of C_1 plants was done using polymorphic SNPs originally used for genotyping the S_2 families of population. Based on the prediction models, the genomic estimated breeding values (GEBVs) of each plant were estimated. A larger GEBV indicated a favourable plant; the top 5% of individuals with high GEBVs in each population were intermated to form the next recombinant cycle 2 (C_2). In the next season, the bulked seeds of C_2 from each population was planted ear-to-row; and similar process followed in C_2 was used to constitute cycle 3 (C_3). Thus, C_1 was constituted based on the phenotypic data, whereas C_2 and C_3 were constituted based on genotypic data. The improved cycles (C_1 , C_2 and C_3) from each population were subjected to doubled haploid production. A total of 111 maize doubled haploids derived from Cycle 1, Cycle 2 and Cycle 3 of MPS 1 (HGA) and MPS 2 (HGB) populations were then crossed to an inbred tester CML451 belonging to heterotic group B and the crossed seeds were harvested for evaluation. These 111 maize DH testcross progenies (Table 2) along with six commercial check hybrids were analysed in the present study for the association of morpho-physiological and yield attributing traits with the grain yield under different temperature regimes.

Table 2. Summary of maize DH testcross progenies (tester CML451, HGB) derived from C_1 , C_2 and C_3 of MPS 1 and MPS 2 populations evaluated under heat stress, optimal and early spring at Bheemaranagudi and Raichur during 2018-19

Sl. No.	Population	Description	DH Testcrosses
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1	MPS-1-C ₁	Testcrosses of DH from Cycle 1 of MPS-1	33
2	MPS-1-C ₂ GS	Testcrosses of DH from Cycle 2 Genomic selection of MPS-1	20
3	MPS-1-C ₃ GS	Testcrosses of DH from Cycle 3 Genomic selection of MPS-1	14
4	MPS-2-C ₁	Testcrosses of DH from Cycle 1 of MPS-2	14
5	MPS-2-C ₂ GS	Testcrosses of DH from Cycle 2 Genomic selection of MPS-2	17
6	MPS-2-C ₃ GS	Testcrosses of DH from Cycle 3 Genomic selection of MPS-2	13
Total			111
Checks : 900MG, DKC9108, NK 6240, P3436, P3550, RCRMH2			

Experimental method

The experimental material was initially evaluated under natural heat stress condition which was achieved by delayed planting in summer, *i.e.*, 2nd fortnight of March 2018. So, that most part of the crop growth including reproductive stage was exposed to high day and night temperature regimes. The trial set was also evaluated during *kharif* 2018, under well-watered condition (optimal) with no exposure to heat stress at any crop stage and under late *rabi* season (early spring) *i.e.*, 1st fortnight of January 2019 at Bheemaranagudi and Raichur, where in post flowering stage of the crop got exposed to natural heat stress. The experimental trials in each season were laid out using alpha lattice design with two replications. Each testcross seeds were hand dibbled in single row of 2.1 m length, with a spacing of 60 × 20 cm. At the time of sowing, the recommended fertilizer dose of 150 : 75 : 37.5 kg NPK ha⁻¹ *i.e.*, the entire dose of phosphorous, potash and 15 kg of nitrogen ha⁻¹ was applied as basal dose and remaining nitrogen was applied in four splits at specific crop stages. The crop was raised by following the recommended agronomic management practices. The moisture stress free situation at any crop growth stage was maintained by providing supplemental irrigation as per the crop requirement to ensure only heat stress as the most limiting factor during trial evaluation. The trials were also kept free from any other biotic or abiotic stresses and the crop growth was satisfactory.

The testcrosses of each MPS populations were evaluated at both locations across three seasons for recording phenotypic data on morpho-physiological, yield and its component traits. From each entry in each replication five competitive plants were randomly selected and tagged for recording of observation on characters *viz.*, SPAD chlorophyll meter reading (SCMR) at pre and post-flowering, NDVI at pre and post-flowering, plant height, cob height, cob length, cob girth, number of kernels per cob, shelling percentage, 100 grain weight and grain yield per plant. The characters *viz.*, days to 50% anthesis, days to 50% silking, days to physiological maturity, leaf firing, tassel blast and grain yield were recorded on plot basis. The grain yield per plot was later expressed as t ha⁻¹ adjusted to 12.5% moisture content. The mean values were computed from individual plants for all the characters and utilized for the statistical analysis.

Statistical analysis

The computed mean data on all the individual characters were subjected to GENSTAT (14.1 edition) software for elucidating the general analysis of variance. The significance of mean squares for the main and interaction effects were tested using the *p*-

value obtained from the same software. The simple correlation coefficients were calculated to determine the degree of associations among different characters and tested against critical values for Pearson's correlation coefficients at $(n - 2)$ degree of freedom both at 0.05 and 0.01 probability levels for their significance. Simple correlations were calculated by using the following formula.

$$\text{Phenotypic correlation} = r(p) = \frac{\text{COV}_{xy}(p)}{\sqrt{V_x(p)V_y(p)}}$$

Where,

- $\text{COV}_{xy}(p)$ = Phenotypic covariance between characters X and Y
- $V_x(p)$ = Phenotypic variance of character X
- $V_y(p)$ = Phenotypic variance of character Y

The META-R (Multi-Environment Trait Analysis with R) software available in CIMMYT repository was used to compute the phenotypic correlation coefficients at different environments and between morpho-physiological, yield and yield attributing traits and their statistical significance was obtained from the same software.

Results and Discussion

Analysis of variance

The Analysis of variance for major morpho-physiological and yield attributing characters evaluated across seasons *viz.*, heat stress, early spring and optimal conditions at Bheemaranagudi and Raichur during 2018-19 are presented in Table 3. The combined ANOVA across locations and environmental situations revealed that, the mean sum of squares due to environment were highly significant for all the characters except anthesis to silking interval and SCMR at pre-flowering, indicating the three imposed environments *viz.*, summer (heat stress), *kharif* (optimal) and late *rabi* (early spring) were different and the characters behaved differently in each seasons. The mean sum of squares due to location was non-significant for all the traits except SCMR at post flowering, NDVI at pre-flowering, days to physiological maturity and grain yield per plant, indicating the two locations were statistically similar because they come under north-eastern dry zone of Karnataka.

The mean squares due to populations were highly significant for all the characters except for plant height and cob height also the mean squares due to cycles were highly significant for all the listed characters except NDVI at post-flowering stage. Thus, the DH based testcrosses in each cycles of MPS 1 and MPS 2 populations were distinct from one another, which indicated the presence of significantly higher amount of genetic variability in the studied material. These results are in agreement with the findings of [22], who reported the mean sum of squares due to genotypes in MPS 1 population differed significantly for grain yield and MPS 2 population showed significant variation among genotypes for all the characters studied across two locations under heat stress condition. [21] also reported that, the mean sum of squares due to genotypes were highly significant for days to 50 per cent anthesis, days to 50 per cent silking, plant height, ear height, ears per plant and grain yield

indicating variability among the families for various traits, for selection under heat stress condition.

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Table 3. Analysis of variance for morphological and yield related characters of maize testcrosses involving DH lines from C₁, C₂ and C₃ of MPS 1 and MPS 2 populations evaluated under heat stress, optimal and early spring at two locations during 2018-19

Source of variation	d.f.	Mean sum of squares						
		Days to 50% anthesis	Days to 50% silking	Anthesis to silking interval	Plant height(cm)	Number of kernels per cob	100 grain weight (g)	Grain yield (t ha ⁻¹)
Replication	1	26.46	70.29	12.30	562.80	31291.00	95.16	34.81
Environment	2	17894.80**	18493.00**	12.84	901190.00**	2031722.00*	22635.60**	2115.72**
Location	1	2370.48	3604.28	129.26	13527.90	952336.00	0.61	694.09
Population	1	994.75**	563.37**	56.80**	413.80	496678.00**	74.17**	181.98**
Cycles	2	851.95**	1020.07**	7.54*	17455.90**	122974.00**	46.47**	98.94**
Env. × Loc.	2	325.78	537.88*	70.07	204440.00**	308867.00**	28.71	71.17**
Env. × Pop.	2	35.47**	21.79*	7.74*	4955.60**	73207.00**	245.07**	12.07**
Loc. × Pop.	1	505.77**	811.94**	39.27**	59614.80**	34167.00**	182.86**	96.45**
Env. × Cyc.	4	55.97**	50.92**	1.03	820.80**	34355.00**	119.24**	20.73**
Loc. × Cyc.	2	241.74**	373.13**	14.68**	19600.90**	181935.00**	15.28	121.55**
Pop. × Cyc.	2	19.80*	30.63**	23.86**	2050.70**	157744.00**	56.17**	21.60**
Env. × Loc. × Pop.	2	24.55**	3.10	13.89**	6957.80**	35010.00**	74.26**	76.43**
Env. × Loc. × Cyc.	4	52.97**	75.68**	4.07	2798.20**	25204.00**	40.18**	18.91**
Env. × Pop. × Cyc.	4	24.02**	22.69**	6.47**	940.10**	62597.00**	69.01**	42.14**
Loc. × Pop. × Cyc.	2	10.24	8.32	5.91*	678.80*	44340.00**	13.16	36.21**
Env. × Loc. × Pop. × Cyc.	4	55.47**	41.92**	2.76	8391.70**	44411.00**	19.67*	8.30**
Error	1290	5.27	5.99	1.93	174.50	4162.00	7.98	1.85
Total	1331							

* and ** - Significant at 0.05 and 0.01 level of probability, respectively.

Table 3. Contd.,

Source of variation	d.f.	Mean sum of squares											
		SCMR-1	SCMR-2	NDVI-1	NDVI-2	LF	TB	CH	DPM	CL	CG	SP	GYP
Replication	1	139.33	494.34**	16.33	0.05	5.99	14.97	3.78	4.71	103.86	59.41	43.18	2091.90
Environment	2	924.09	6301.58**	5230.58**	1.32*	–	–	343571.45**	24896.07**	303.43*	421.78*	32793.29**	744960.60**
Location	1	8805.61	18023.89**	11859.66**	5.56	0.01	685.23	25623.79	3127.62*	874.75	263.63	2577.25	316630.40*
Population	1	1018.87**	1495.85**	1062.11**	1.19**	7.89**	41.13**	96.74	410.88**	20.33**	142.53**	526.22**	68376.60**
Cycles	2	755.19**	480.87**	287.66**	0.01	10.31**	111.96**	4590.02**	112.41**	40.33**	23.08**	121.13*	27987.20**
Env. × Loc.	2	7144.90**	13179.12**	2487.01	1.32*	–	–	97431.11	146.15**	8.24	34.92	466.92	36930.00
Env. × Pop.	2	25.91	49.43**	150.26**	0.20**	–	–	1143.73**	1.85	22.80**	6.96**	544.01**	5117.40**
Loc. × Pop.	1	191.39**	7.65	54.37	0.08**	2.69**	71.57**	16309.24**	148.74**	66.09**	29.10**	1450.58**	23123.50**
Env. × Cyc.	4	95.88**	52.88**	150.09**	0.03**	–	–	9.86	3.73	17.30**	4.39**	327.30**	7294.00**
Loc. × Cyc.	2	85.85**	28.07	543.12**	0.01	2.08*	100.58**	5090.37**	58.70**	32.05**	8.16**	620.51**	32564.70**
Pop. × Cyc.	2	4.66	34.00**	6.33	0.01	1.63	27.39**	492.35**	94.33**	23.43**	17.93**	321.16**	9113.00**
Env. × Loc. × Pop.	2	13.43	43.39**	86.25**	0.09**	–	–	2542.41**	8.69	23.22**	8.25**	147.86**	18585.10**
Env. × Loc. × Cyc.	4	54.25**	56.53**	29.63	0.03**	–	–	253.80*	5.74	7.62*	9.95**	61.55	5349.50**
Env. × Pop. × Cyc.	4	98.65**	58.94**	32.37	0.11**	–	–	376.48**	2.50	13.88**	11.76**	19.17	12434.80**
Loc. × Pop. × Cyc.	2	241.16**	49.34**	185.98**	0.04**	0.35	28.22**	513.79**	92.34**	23.35**	8.25**	170.54**	10257.00**
Env. × Loc. × Pop. × Cyc.	4	63.73**	164.70**	130.03**	0.005	–	–	1797.97**	2.55	5.91	4.99**	131.02**	3458.90**
Error	1290	11.37	10.64	27.20	0.01	0.64	1.89	84.86	6.97	2.87	0.68	30.86	681.60**
Total	1331												

* and ** - Significant at 0.05 and 0.01 level of probability, respectively. SCMR-1 = SCMR pre-flowering, SCMR-2 = SCMR post-flowering, NDVI-1 = NDVI pre-flowering, NDVI-2 = NDVI post-flowering, LF = Leaf firing (%), TB = Tassel blast (%), CH = Cob height (cm), DPM = Days to physiological maturity, CL = Cob length (cm), CG = Cob girth (cm), SP = Shelling percentage and GYP = Grain yield per plant (g).

Association of traits in maize testcrosses under heat stress condition

In the present investigation, the correlation coefficients of maize testcrosses involving DH lines from C₁, C₂ and C₃ of MPS 1 and MPS 2 populations evaluated over two locations under heat stress condition for all the 18 characters are presented in Table 4. The grain yield per plant showed positive significant association with number of kernels per cob ($r = 0.801$) followed by cob girth ($r = 0.647$), SCMR pre-flowering ($r = 0.443$), cob length ($r = 0.417$), NDVI post-flowering ($r = 0.395$), SCMR post-flowering ($r = 0.354$), plant height ($r = 0.347$), cob height ($r = 0.291$), shelling percentage ($r = 0.224$) and NDVI pre-flowering ($r = 0.201$). These significant correlations with grain yield indicated the dependence of these characters on each other, which means grain yield per plant increases with increase in value of these characters and *vice-versa*. Hence, simultaneous selection criteria should be considered for those traits which showed strong correlation and that might bring an improvement in grain yield of maize. The similar results were reported by [16], [17], [18], [19] and [14] under heat stress condition. [20] reported significant positive association of grain yield per plant with the traits like plant height, cob length, cob girth, chlorophyll content at 55 days and NDVI at 55 days under heat stress condition. [21] also reported significant positive association of grain yield per plant with the plant height and cob height under heat stress situation.

The significant and negative correlation (Table 4) was exhibited by days to 50% anthesis ($r = -0.271$) and days to 50% silking ($r = -0.297$) indicating the earlier tasseling and silking days are related with high grain yield in maize under heat stress. Similar findings were reported by [19], [14] and [20], where the traits days to 50% anthesis and days to 50% silking showed significant negative association with grain yield per plant under heat stress condition. Further, 100 grain weight ($r = 0.141$), days to physiological maturity ($r = 0.105$), anthesis to silking interval ($r = 0.018$), tassel blast ($r = -0.026$) and leaf firing ($r = -0.098$) exhibited negative and non-significant association with yield (Table 4). This was in accordance with results of [1], [18] and [14], they reported that anthesis-silking interval, leaf firing and tassel blast showed negative and non-significant relationship with yield under heat stress condition and recommended to consider in index of selection for heat stress tolerance in tropical maize.

The report by [23] suggests that, if the morphological traits which do not show any significant association or very negligible amount of association, those can be discarded to reduce the number of traits to be characterized. Elimination of excessive traits will not only reduce the workload of researcher but also will make characterization less cumbersome and more efficient. Whereas, this conclusion may not be valid as non-significant association does not necessarily mean that the traits are not biologically related and might probably be a result of decreased phenotypic variation among the genotypes for this trait under heat stress [14].

Association of traits in maize testcrosses under optimal condition

The results pertaining to correlation coefficients of DH testcrosses resulted from C₁, C₂ and C₃ of MPS 1 and MPS 2 populations evaluated over two locations under optimal condition (*kharif*) for 16 characters of maize are presented in Table 5. The grain yield per

plant showed highly significant association with traits viz., number of kernels per cob ($r = 0.719$), cob girth ($r = 0.600$), cob length ($r = 0.516$), SCMR post-flowering ($r = 0.325$), plant

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Table 4. Phenotypic correlation coefficients for various characters of maize testcrosses involving DH lines from C₁, C₂ and C₃ of MPS 1 and MPS 2 populations evaluated under heat stress (summer) across two locations during 2018

Trait	AD	SD	ASI	SCMR-1	SCMR-2	NDVI-1	NDVI-2	LF	TB	PH	CH	DPM	CL	CG	NKC	100GW	SP
SD	0.920**																
ASI	-0.315**	0.069															
SCMR-1	-0.180*	-0.239**	-0.109														
SCMR-2	0.095	0.039	-0.167	0.721**													
NDVI-1	0.439**	0.371**	-0.214**	0.136	0.308**												
NDVI-2	0.252**	0.178*	-0.160	0.223**	0.279**	0.486**											
LF	-0.108	-0.122	-0.065	-0.052	0.001	-0.146	-0.205*										
TB	-0.210*	-0.161	0.104	0.008	-0.006	-0.072	-0.168	0.524**									
PH	0.133	0.067	-0.204*	0.213*	0.322**	0.302**	0.345**	0.032	0.087								
CH	0.192*	0.127	-0.211*	0.193*	0.329**	0.360**	0.271*	-0.023	0.090	0.753**							
DPM	0.201*	0.186*	-0.077	0.060	0.127	0.201*	0.075	-0.048	0.059	0.210*	0.102						
CL	-0.185*	-0.233**	-0.068	0.320**	0.159	0.109	0.213*	-0.051	0.029	0.293**	0.253**	0.103					
CG	-0.058	-0.117	-0.074	0.361**	0.312**	0.306**	0.314**	-0.154	0.050	0.312**	0.248**	0.091	0.227**				
NKC	-0.295**	-0.340**	-0.006	0.339**	0.267**	0.160	0.368**	-0.166	-0.037	0.317**	0.286**	0.014	0.426**	0.681**			
100GW	0.004	0.029	0.020	0.249**	0.286**	0.105	0.153	0.097	0.139	0.182*	0.150	0.083	0.219*	0.032	-0.098		
SP	-0.405**	-0.399**	0.076	0.094	0.008	-0.162	-0.031	-0.053	0.117	0.107	0.070	-0.022	0.152	0.217*	0.404**	-0.002	
GYP	-0.271**	-0.297**	0.018	0.443**	0.354**	0.201*	0.395**	-0.098	-0.026	0.347**	0.291**	0.105	0.417**	0.647**	0.801**	0.141	0.224**

“* and **” - Significant at 0.05 and 0.01 level of probability, respectively. AD = Days to 50% anthesis, SD = Days to 50% silking, ASI = Anthesis to silking interval, SCMR-1 = SCMR pre-flowering, SCMR-2 = SCMR post-flowering, NDVI-1 = NDVI pre-flowering, NDVI-2 = NDVI post-flowering, LF = Leaf firing (%), TB = Tassel blast (%), PH = Plant height (cm), CH = Cob height (cm), DPM = Days to physiological maturity, CL = Cob length (cm), CG = Cob girth (cm), NKC = Number of kernels per cob, 100GW = 100 Grain weight (g), SP = Shelling percentage (%) and GYP = Grain yield per plant (g)

Table 5. Phenotypic correlation coefficients for various characters of maize testcrosses involving DH lines from C₁, C₂ and C₃ of MPS 1 and MPS 2 populations evaluated under optimal condition (*kharif*) across two locations during 2018

Trait	AD	SD	ASI	SCMR-1	SCMR-2	NDVI-1	NDVI-2	PH	CH	DPM	CL	CG	NKC	100GW	SP
SD	0.861**														
ASI	-0.075	0.423**													
SCMR-1	-0.171*	-0.294**	-0.237**												
SCMR-2	-0.125	-0.245**	-0.222**	0.800**											
NDVI-1	0.073	0.064	-0.018	0.017	-0.060										
NDVI-2	-0.056	-0.145	-0.213*	0.328**	0.320**	0.124									
PH	-0.038	-0.152	-0.175*	0.185*	0.310**	-0.066	0.233**								
CH	0.206*	0.150	-0.038	0.002	0.168	-0.100	0.074	0.715**							
DPM	0.346**	0.331**	0.026	-0.052	0.084	0.048	-0.010	0.049	0.163						
CL	0.005	-0.133	-0.270**	0.138	0.154	-0.079	0.150	0.306**	0.278**	0.008					
CG	0.112	-0.062	-0.296**	0.233**	0.249**	-0.073	0.167	0.278**	0.308**	0.009	0.258**				
NKC	-0.141	-0.309**	-0.387**	0.243**	0.262**	-0.058	0.241**	0.207*	0.131	-0.015	0.532**	0.622**			
100GW	0.015	-0.040	-0.071	0.010	-0.032	0.120	0.082	0.158	0.185*	0.120	0.059	0.078	-0.285**		
SP	-0.362**	-0.347**	-0.059	0.065	-0.132	-0.087	0.140	-0.101	-0.174*	-0.423**	0.029	-0.156	0.114	-0.099	
GYP	-0.038	-0.278**	-0.471**	0.282**	0.325**	0.003	0.275**	0.293**	0.272**	0.110	0.516**	0.600**	0.719**	0.099	-0.051

“* and **” - Significant at 0.05 and 0.01 level of probability, respectively. AD = Days to 50% anthesis, SD = Days to 50% silking, ASI = Anthesis to silking interval, SCMR-1 = SCMR pre-flowering, SCMR-2 = SCMR post-flowering, NDVI-1 = NDVI pre-flowering, NDVI-2 = NDVI post-flowering, PH = Plant height (cm), CH = Cob height (cm), DPM = Days to physiological maturity, CL = Cob length (cm), CG = Cob girth (cm), NKC = Number of kernels per cob, 100GW = 100 Grain weight (g), SP = Shelling percentage (%) and GYP = Grain yield per plant (g)

height ($r = 0.293$), SCMR pre-flowering ($r = 0.282$), NDVI post-flowering ($r = 0.275$) and cob height ($r = 0.272$), indicated the dependence of these characters on each other and grain yield per plant would increase with increase in value of these characters and *vice-versa*. This was supported by the earlier reports by [14], who reported that the grain yield per plant of hybrids under optimal conditions showed positive and significant association with the traits *viz.*, days to 50% anthesis, days to 50% silking, cob girth, plant height and kernels rows per cob. Similarly, [20] also reported the traits plant height, cob length, cob girth, 100 grain weight and days to 50% silking exhibited positively significant correlation with grain yield per plant.

In contrary, the traits *viz.*, days to 50% silking ($r = -0.278$) and anthesis to silking interval ($r = -0.471$) showed significant correlation coefficient with grain yield per plant but in a negative direction, which indicated that earlier tasseling and minimum anthesis to silking interval might contribute to high grain yield (Table 5). Similar findings were reported by [20], who found negative and significant association between grain yield per plant and anthesis to silking interval under optimal condition. On the other hand, days to 50% anthesis, NDVI pre-flowering, days to physiological maturity, 100 grain weight and shelling percentage exhibited non-significant association with grain yield per plant indicating the independent expression of characters and may not contribute towards increase in grain yield (Table 5). Similar findings was reported by [20], who found non-significant association of days to 50% anthesis, NDVI at 55 and 82 days with grain yield per plant under optimal condition. [24]and[25] found non-significant correlation between grain yield per plant and 100 grain weight and [26] reported non-significant association of shelling percentage with grain yield per plant under *kharif* season.

Association of traits in maize testcrosses under early spring condition

The association of traits in yield and other yield related traits of maize testcrosses involving DH lines from C₁, C₂ and C₃ of MPS 1 and MPS 2 populations evaluated over two locations under early spring (late *rabi*) condition are presented in Table 6. The grain yield per plant was positively and significantly associated with number of kernels per cob ($r = 0.701$) followed by cob length ($r = 0.630$), cob girth ($r = 0.571$), 100 grain weight ($r = 0.559$), SCMR post-flowering ($r = 0.414$), plant height ($r = 0.375$), shelling percentage ($r = 0.335$), NDVI post-flowering ($r = 0.294$), SCMR pre-flowering ($r = 0.252$), days to physiologicalmaturity ($r = 0.207$) and cob height ($r = 0.135$) which indicated the dependence of these characters on each other and grain yield per plant increases with increase in value of these characters and would suggest indirect selection of genotypes for grain yield. The similar results also reported by [27], who reported that, under summer and winter season the traits *viz.*, plant height, cob height, cob length, cob girth, number of kernels and rows per cob and 100 grain weight showed positive and significant association with grain yield. The flowering traits (Table 6) like days to 50% anthesis, days to 50% silking, anthesis to silking interval and NDVI pre-flowering showed non-significant association with grain yield per plant, which indicated that, these characters were independent and may not contribute to increase in grain yield. Likewise, [28]reported that the grain yield exhibited non-significant correlation with days to 50% anthesis, days to 50% silking and anthesis to silking interval in winter maize.

Table 6. Phenotypic correlation coefficients for various characters of maize testcrosses involving DH lines from C₁, C₂ and C₃ of MPS 1 and MPS 2 populations evaluated under early spring (late *rabi*) condition across two locations during 2018–19

Trait	AD	SD	ASI	SCMR-1	SCMR-2	NDVI-1	NDVI-2	PH	CH	DPM	CL	CG	NKC	100GW	SP
SD	0.921**														
ASI	0.041	0.415**													
SCMR-1	-0.229**	-0.234**	-0.099												
SCMR-2	-0.051	-0.112	-0.196*	0.518**											
NDVI-1	-0.030	0.025	0.054	0.217*	0.155										
NDVI-2	0.298**	0.205*	0.171*	0.013	0.228*	0.100									
PH	0.051	0.040	-0.069	0.165	0.255**	0.246**	0.316**								
CH	0.193*	0.150	-0.087	0.021	0.083	0.212*	0.250**	0.628**							
DPM	0.120	0.064	-0.119	0.039	0.258**	0.133	0.222**	0.241**	0.301**						
CL	-0.180*	-0.164	-0.013	0.135	0.320**	0.156	0.174*	0.334**	0.014	0.169*					
CG	0.076	0.100	0.044	0.157	0.336**	0.067	0.335**	0.210*	-0.078	0.038	0.425**				
NKC	-0.178*	-0.164	-0.052	0.251**	0.363**	0.197*	0.205*	0.351**	0.026	0.153	0.700**	0.635**			
100GW	0.051	0.009	-0.101	0.086	0.293**	-0.022	0.309**	0.304**	0.210*	0.198*	0.343**	0.235**	0.097		
SP	-0.299**	-0.316**	-0.123	0.213*	0.216*	0.142	-0.018	0.161	0.075	0.100	0.434**	0.166	0.597**	0.127	
GYP	-0.106	-0.130	-0.128	0.252**	0.414**	0.082	0.294**	0.375**	0.135*	0.207*	0.630**	0.571**	0.701**	0.559**	0.335**

“* and **”- Significant at 0.05 and 0.01 level of probability, respectively. AD = Days to 50% anthesis, SD = Days to 50% silking, ASI = Anthesis to silking interval, SCMR 1 = SCMR pre-flowering, SCMR-2 = SCMR post-flowering, NDVI-1 = NDVI pre-flowering, NDVI-2 = NDVI post-flowering, PH = Plant height (cm), CH = Cob height (cm), DPM = Days to physiological maturity, CL = Cob length (cm), CG = Cob girth (cm), NKC = Number of kernels per cob, 100GW = 100 Grain weight (g), SP = Shelling percentage (%) and GYP = Grain yield per plant (g)

In the present study, the phenotypic correlation analysis considering across heat stress, early spring and optimal conditions revealed that, the grain yield per plant showed desirable (positive and significant) association with the traits *viz.*, number of kernels per cob, cob length and cob girth; whereas, plant height, cob height, SCMR at pre and post-flowering and NDVI at post flowering showed significant weak association with grain yield per plant under all the seasons, suggest the presence of common genetic factors controlling expression of these traits under all the environments. Therefore, the selection of genotypes having higher number of kernels per cob, cob length and cob girth should be the priority for breeders to achieve higher yields in maize. The number of kernels per cob showed very strong significant correlation with grain yield per plant under heat stress condition indicating that, the genotype which produces higher number of kernels per cob could be selected for higher grain yield because the kernel numbers tend to decrease under heat stress. In addition, days to 50% silking showed significant negative correlation with grain yield per plant under heat stress and optimal condition indicated that, the genotypes which exhibit earlier silking might contribute to high grain yield. Hence, the genotypes with earliness in silking should be the priority for improving the higher grain yield in maize under heat stress condition.

In this investigation, we did not found any significant association of anthesis-silking interval with grain yield per plant under heat stress and early spring conditions, but found negative significant association during optimal condition; this may be due to phenotypic plasticity of individual genotypes. However, this trait has the potential to improve the selection efficiency for grain yield under stress environments. Several earlier reports have also indicated the effectiveness of flowering traits, especially anthesis to silking interval in the selection for tolerance under stress environments [29, 30].

Conclusion

Findings of the study are the first of its kind to report the character association between different morpho-physiological, yield and yield attributing traits under three environmental situations *viz.*, heat stress, optimal and early spring conditions across two locations. Therefore, it is concluded that the genotypes which exhibit earlier silking, higher number of kernels per cob, cob length and cob girth might contribute to higher grain yield. Hence, the simultaneous selection criteria for these traits should be the priority for improving the higher grain yield in maize under heat stress condition as well as optimal condition.

Data Availability: All relevant data are within the paper

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