Plant Archives Vol. 24, No. 2, 2024 pp. 2700-2714 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.2.386

GENETIC GAINS FOR HEAT STRESS TOLERANCE IN MAIZE THROUGH INTEGRATION OF RAPID CYCLE GENOMIC SELECTION AND DOUBLED HAPLOID TECHNOLOGY

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(Date of Receiving-26-05-2024; Date of Acceptance-12-08-2024)

ABSTRACT

Rapid cycle genomic selection (RCGS) has enormous potential to increase the genetic gains by shortening the breeding cycle and reducing phenotyping costs. Doubled haploid (DH) technology allows production of inbred lines in two generations. Hence, the study was designed to assess the genetic gain of DH hybrids derived from improved cycles of multi-parental synthetic (MPS) population 1 and 2, developed through the integration of genomic selection and DH technology. The experiment involved evaluation of 111 DH testcross hybrids derived from improved cycles C_1, C_2 and C_3 across two locations Bheemarayanagudi and Raichur in Karnataka during 2018 and 2019 under heat stress, early spring and optimal growing conditions. The genetic gain analysis showed that, under optimal condition, testcrosses of C_3 in MPS 2 registered marginal genetic gain over C_1 and C_2 with nominal yield gain of 58 kg ha^{-1} $cycle^{-1}$. In early spring, C_2 testcrosses of MPS 2 showed genetic gain over C_1 and across cycles 29 kg ha^{-1} $cycle^{-1}$ yield gain was noticed. Whereas under heat stress condition, the MPS 2 did not show any genetic gain over cycles. However, the MPS 1 population showed increased grain yield from C_1 to C_2 then yield got decreased from C_2 to C_3 resulted in yield penalty of 397 kg ha^{-1} $cycle^{-1}$, 72 kg ha^{-1} $cycle^{-1}$ and 393 kg ha^{-1} $cycle^{-1}$ under optimal, early spring and heat stress condition, respectively. Thus, MPS 1 and MPS 2 populations showed a differential response to rapid cycle genomic selection under different seasons. Further, the RCGS has simultaneously improved the key traits, anthesis to silking interval, leaf firing and tassel blast traits along with the grain yield in both populations.

Key words : Maize, Heat stress, Rapid cycle genomic selection, Doubled haploid, Genetic gain.

Introduction

Climate change and population growth pose significant challenges to food security for millions of people, particularly in sub-Saharan Africa, Asia and Latin America. In this relation, maize as a major food security and income-generating crop plays a critical role in ensuring food security, nutritional security and livelihoods for resource-constrained smallholders in these regions (Prasanna *et al.*, 2021). Maize is mainly grown in warmer temperate regions and humid subtropical climates and is highly productive under optimal environmental and crop management conditions. But, climate-induced stresses like drought, waterlogging, salinity, heat, cold, diseases and pests can severely impact production. Heat stress is another major constraint to maize production, as the rise in temperature beyond threshold level can cause irreversible damage to crop growth and yield. Maize is highly vulnerable to reproductive stage drought or high temperature stress. The most crop growth period of spring maize in Asian tropics grown during February – May is invariably exposed to high temperature regimes starting from late vegetative stage until early grain filling stage (Zaidi *et al.*, 2020). A study on the impact of current and future heat stress on maize in South Asia has shown that areas affected by heat stress will significantly increase in the future, particularly in the pre-monsoon (spring) and monsoon (rainy) seasons (Tesfaye *et al.*, 2017). The study also highlighted the potential yield advantage of heat tolerant maize varieties in both spring and rainy seasons, relative to current heat-vulnerable maize varieties that are extensively grown in the region.

In order to expedite the development of cultivars for climate resilience in a quick time, there is a need of modern breeding tools like genomic selection and double haploid (DH) technology in a maize breeding programme. Genomic selection or rapid cycle genomic selection (RCGS) reduces length of the breeding cycle to a single season, as it involves selection and intermating based on genomic estimated breeding value (GEBV), thereby one can get increased genetic gain. Recent studies on GEBV enabled selection of superior phenotypes in maize resulted the rapid genetic gain for drought (Beyene *et al.*, 2015; Vivek *et al.*, 2017; Zhang *et al.*, 2017 and Das *et al.*, 2020) and for heat tolerance (Hosmani *et al.*, 2020 and Swamy *et al.*, 2023). Similarly, DH technology has become an integral part and an efficient alternative of many commercial maize breeding programmes to generate 100% homozygous lines in quick time-frame (two generations), which otherwise takes longer time in the conventional inbreeding procedure (Chaikam *et al.*, 2019). These 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. Keeping these facts in background, the study was designed with an objective to assess the genetic gain in DH testcrosses derived from improved cycles. In this research article, we are reporting the genetic gain in testcrosses involving DH lines from improved cycles of MPS 1 and MPS 2 populations developed through RCGS for heat stress tolerance/resilience.

Materials and Methods

Experimental location

The present experiment was laid out (Table 1) during 2018 (summer and *kharif*) and 2019 (late *rabi*) at Agriculture College Farm, Bheemarayanagudi situated at 16^o 72' N Latitude, 76^o 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^o 19' N Latitude, 77^o 31' E.

Longitude with an altitude of 407 m above M.S.L. Bheemarayanagudi and Raichur come under north-eastern dry zone of Karnataka, India. The weather data (Table 1) 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 ($T_{max.} > 35$ °C and $T_{min.} \geq 21$°C) and relative humidity (< 50%) ensured the proper evaluation of trial. Therefore, the warm and dry humid climatic conditions of the locations were appropriate to evaluate 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, which 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 progenies 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 the C_1 plants was done using the polymorphic SNPs originally used for genotyping the S_2 families of the population. Based on the prediction models, the genomic estimated breeding values (GEBVs) of each plant were estimated. 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

Table 1 : Details of locations and environments used for evaluation of maize testcrosses involving DH lines from C_1, C_2 and C_3 of MPS 1 and MPS 2 populations and checks during 2018 and 2019.

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
			Kharif 2018 (August-November)	Optimal	Rain-fed with protective irrigation	20.60	33.33	118.60	66.23
			Late *rabi* 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
			Kharif 2018 (August-November)	Optimal	Rain-fed with protective irrigation	22.10	33.05	160.40	61.00
			Late *rabi* 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

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 genetic gain analysis under different temperature regimes.

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 evaluated during *kharif* 2018, under well-watered condition (optimal) with no exposure to heat stress at any crop stage. Later, same set was also evaluated under late *rabi* season (early spring) *i.e.*, 1st fortnight of January 2019 at Bheemarayanagudi 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

Table 2 : List 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 Bheemarayanagudi and Raichur during 2018 and 2019.

M.P.S. 1 population (HGA)			
S. no.	**Pedigree**	**S. no.**	**Pedigree**
1	CML451/(MPS-1-C1)-DH1	34	CML451/(MPS-1-C2GS)-DH2
2	CML451/(MPS-1-C1)-DH3	35	CML451/(MPS-1-C2GS)-DH5
3	CML451/(MPS-1-C1)-DH9	36	CML451/(MPS-1-C2GS)-DH7
4	CML451/(MPS-1-C1)-DH11	37	CML451/(MPS-1-C2GS)-DH12
5	CML451/(MPS-1-C1)-DH12	38	CML451/(MPS-1-C2GS)-DH16
6	CML451/(MPS-1-C1)-DH24	39	CML451/(MPS-1-C2GS)-DH21
7	CML451/(MPS-1-C1)-DH26	40	CML451/(MPS-1-C2GS)-DH22
8	CML451/(MPS-1-C1)-DH27	41	CML451/(MPS-1-C2GS)-DH25
9	CML451/(MPS-1-C1)-DH28	42	CML451/(MPS-1-C2GS)-DH29
10	CML451/(MPS-1-C1)-DH30	43	CML451/(MPS-1-C2GS)-DH30
11	CML451/(MPS-1-C1)-DH34	44	CML451/(MPS-1-C2GS)-DH33
12	CML451/(MPS-1-C1)-DH36	45	CML451/(MPS-1-C2GS)-DH37
13	CML451/(MPS-1-C1)-DH38	46	CML451/(MPS-1-C2GS)-DH40
14	CML451/(MPS-1-C1)-DH40	47	CML451/(MPS-1-C2GS)-DH42
15	CML451/(MPS-1-C1)-DH41	48	CML451/(MPS-1-C2GS)-DH45
16	CML451/(MPS-1-C1)-DH45	49	CML451/(MPS-1-C2GS)-DH56
17	CML451/(MPS-1-C1)-DH46	50	CML451/(MPS-1-C2GS)-DH61
18	CML451/(MPS-1-C1)-DH47	51	CML451/(MPS-1-C2GS)-DH64
19	CML451/(MPS-1-C1)-DH49	52	CML451/(MPS-1-C2GS)-DH67
20	CML451/(MPS-1-C1)-DH50	53	CML451/(MPS-1-C2GS)-DH70
21	CML451/(MPS-1-C1)-DH55	54	CML451/(MPS-1-C3GS)-DH2
22	CML451/(MPS-1-C1)-DH58	55	CML451/(MPS-1-C3GS)-DH3
23	CML451/(MPS-1-C1)-DH59	56	CML451/(MPS-1-C3GS)-DH5
24	CML451/(MPS-1-C1)-DH60	57	CML451/(MPS-1-C3GS)-DH8
25	CML451/(MPS-1-C1)-DH61	58	CML451/(MPS-1-C3GS)-DH15
26	CML451/(MPS-1-C1)-DH64	59	CML451/(MPS-1-C3GS)-DH18
27	CML451/(MPS-1-C1)-DH65	60	CML451/(MPS-1-C3GS)-DH19
28	CML451/(MPS-1-C1)-DH66	61	CML451/(MPS-1-C3GS)-DH29
29	CML451/(MPS-1-C1)-DH68	62	CML451/(MPS-1-C3GS)-DH30
30	CML451/(MPS-1-C1)-DH69	63	CML451/(MPS-1-C3GS)-DH31
31	CML451/(MPS-1-C1)-DH72	64	CML451/(MPS-1-C3GS)-DH34
32	CML451/(MPS-1-C1)-DH74	65	CML451/(MPS-1-C3GS)-DH38
33	CML451/(MPS-1-C1)-DH75	66	CML451/(MPS-1-C3GS)-DH49
		67	CML451/(MPS-1-C3GS)-DH64
M.P.S. 2 population (HGB)			
S. no.	**Pedigree**	**S. no.**	**Pedigree**
68	CML451/(MPS-2-C1)-DH8	90	CML451/(MPS-2-C2GS)-DH31
69	CML451/(MPS-2-C1)-DH14	91	CML451/(MPS-2-C2GS)-DH42
70	CML451/(MPS-2-C1)-DH15	92	CML451/(MPS-2-C2GS)-DH48
71	CML451/(MPS-2-C1)-DH16	93	CML451/(MPS-2-C2GS)-DH49
72	CML451/(MPS-2-C1)-DH29	94	CML451/(MPS-2-C2GS)-DH57
73	CML451/(MPS-2-C1)-DH36	95	CML451/(MPS-2-C2GS)-DH60
74	CML451/(MPS-2-C1)-DH37	96	CML451/(MPS-2-C2GS)-DH65
75	CML451/(MPS-2-C1)-DH40	97	CML451/(MPS-2-C2GS)-DH74

Table 2 continued...

Table 2 continued...

S. no.	Pedigree	S. no.	Pedigree
76	CML451/(MPS-2-C1)-DH44	98	CML451/(MPS-2-C2GS)-DH75
77	CML451/(MPS-2-C1)-DH47	99	CML451/(MPS-2-C3GS)-DH1
78	CML451/(MPS-2-C1)-DH53	100	CML451/(MPS-2-C3GS)-DH10
79	CML451/(MPS-2-C1)-DH56	101	CML451/(MPS-2-C3GS)-DH11
80	CML451/(MPS-2-C1)-DH70	102	CML451/(MPS-2-C3GS)-DH13
81	CML451/(MPS-2-C1)-DH73	103	CML451/(MPS-2-C3GS)-DH22
82	CML451/(MPS-2-C2GS)-DH1	104	CML451/(MPS-2-C3GS)-DH26
83	CML451/(MPS-2-C2GS)-DH2	105	CML451/(MPS-2-C3GS)-DH30
84	CML451/(MPS-2-C2GS)-DH3	106	CML451/(MPS-2-C3GS)-DH35
85	CML451/(MPS-2-C2GS)-DH9	107	CML451/(MPS-2-C3GS)-DH39
86	CML451/(MPS-2-C2GS)-DH15	108	CML451/(MPS-2-C3GS)-DH45
87	CML451/(MPS-2-C2GS)-DH19	109	CML451/(MPS-2-C3GS)-DH50
88	CML451/(MPS-2-C2GS)-DH20	110	CML451/(MPS-2-C3GS)-DH62
89	CML451/(MPS-2-C2GS)-DH28	111	CML451/(MPS-2-C3GS)-DH68

Summary of DH testcrosses involving different cycles of MPS populations

S. no.	Population	Description	DHTCs
1	MPS-1-C_1	TCs of DH from Cycle 1 of MPS-1	33
2	MPS-1-C_2GS	TCs of DH from Cycle 2 Genomic selection of MPS-1	20
3	MPS-1-C_3GS	TCs of DH from Cycle 3 Genomic selection of MPS-1	14
4	MPS-2-C_1	TCs of DH from Cycle 1 of MPS-2	14
5	MPS-2-C_2GS	TCs of DH from Cycle 2 Genomic selection of MPS-2	17
6	MPS-2-C_3GS	TCs of DH from Cycle 3 Genomic selection of MPS-2	13
Total			**111**

Checks : 900MG, DKC9108, NK 6240, P3436, P3550, RCRMH2

plants were randomly selected and tagged for recording of observation on quantitative characters *viz.*, 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. By utilising the META-R (Multi-Environment Trait Analysis with R) software available in CIMMYT repository the Best Linear and Unbiased Predictors (BLUPs) for various primary and secondary traits were estimated. To estimate per cent genetic gain over cycles, the average of top 10 testcross BLUP values from each cycles C_1, C_2 and C_3 of MPS 1 and MPS 2 populations, at each situation (heat stress, early spring and optimal) and at individual locations (Bheemarayanagudi and Raichur) were utilized for calculation by using the following formula.

$$\text{Per cent gain from } C_1 \text{ to } C_2 = \frac{C_2 - C_1}{C_1} \times 100$$

$$\text{Per cent gain from } C_2 \text{ to } C_3 = \frac{C_3 - C_2}{C_2} \times 100$$

$$\text{Per cent gain from } C_1 \text{ to } C_3 = \frac{C_3 - C_1}{C_1} \times 100$$

Where, C_1 = Cycle 1, C_2 = Cycle 2 and C_3 = Cycle 3

Average gain per cycle or genetic gain response was assessed by regressing the mean values of each cycles (C_1, C_2 and C_3) for each population, each location and at each situations and also across situations for grain yield and other characters.

Results and Discussion

Analysis of variance

The combined ANOVA across locations and environmental situations (Table 3) revealed that, the mean sum of squares due to environment were highly significant for all the characters except anthesis to silking interval, 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, 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 also the mean squares due to cycles were highly significant for all the listed characters. 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 Hosamani *et al*. (2020), 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.

Assessment of genetic gain

Genetic gain for mean grain yield

Anassessment of relative grain yield (t ha$^{”1}$) of testcrosses derived from different GS recombination cycles showed varied responses under different temperature regimes (Table 4). The performance under optimal condition across locations revealed that, the testcrosses of C_3 in MPS 2 population had registered marginal genetic gain over C_1 (1.50%) and C_2 (0.50%), whereas MPS 1 showed 1.48% increased grain yield from C_1 to C_2 then the grain yield got decreased from C_2 to C_3 (–10.96%). The mean of cycles in both populations was higher than the average of checks. The regression analysis showed the nominal positive gain of 58 kg ha$^{”1}$ cycle$^{–1}$ in MPS 2 but 397 kg ha$^{–1}$ cycle$^{–1}$ yield penalty was recorded in MPS 1 population due to reduced grain yield from C_2 to C_3 across locations. Under early spring condition, MPS 1 and MPS 2 populations across locations showed nominal increase in yield from C_1 to C_2 (1.47% and 4.11%, respectively) and nominal yield loss from C_2 to C_3. Thus, the testcrosses of C_2 in MPS 2 produced significantly higher grain yield than MPS 1 and checks. This trend was eventually reflected in the final gain across cycles as well, *i.e*., MPS 2 showed 29 kg ha$^{–1}$ yield gain per cycle, whereas MPS 1 had yield penalty of 72 kg ha$^{–1}$ cycle$^{–1}$. In case of heat stress situation across locations, the MPS 1 showed increased grain yield from C_1 to C_2 (9.30%) and yield loss from C_2 to C_3 (–23.15%) and C_1 to C_3 (–16%), that resulted in 393 kg h^{-1} yield penalty per selection cycle; whereas MPS 2 did not show any yield gain from C_1 to C_2 or C_2 to C_3 and registered 702 kg h^{-1} yield penalty per selection cycle across two locations.

If we consider across three situations (heat stress, early spring and optimal condition) and across heat stress (heat stress and early spring), the gains in grain yield over selection cycles registered consistent genetic gain from C_1 to C_2 in MPS 1 and thereafter there was no gain from C_2 to C_3 or C_1 to C_3 (Table 4). Whereas, the MPS 2 showed differential response at each seasons or no response to selection *i.e*., decreased genetic gain from C_1 to C_2, C_2 to C_3 and C_1 to C_3 both at across three seasons and across heat stress situation and due to this yield penalty was observed across cycles. Das *et al*. (2020) also found the differential response between the MYS 1 and 2 populations to RCGS under drought and waterlogging tolerance and suggested that the strength of lines/trait donors involved in constituting the base population plays a key role in the genetic gain with GS. Therefore, it would be more efficient to evaluate the population per se at the initial stage and move forward with selected potential population for rapid cycling using GS to save on genotyping costs.

The genetic gain in MPS 2 population was observed till C_2 at across locations for early spring and at Raichur under heat stress condition (Table 4); further, the increased genetic gain from C_1 to C_3 at Raichur under optimal condition was noticed, whereas no response to selection was observed at Bheemarayanagudi under heat stress and optimal condition. This may be because, at initial stages of selection and formation of training population, some of the entries were also selected from Raichur based on grain yield. Therefore, it is inferred that, while selection of material and during deployment of tested material care should be taken about testing location. These results were in accordance with findings of Das *et al*. (2020), as they found relatively higher gain between C_2 and C_3 than between C_1 and C_2 in MYS-2 population under drought stress. Beyene *et al*. (2015) reported little or no response to genomic selection from C_1 to C_2 and gain was observed from C_2 to C_3 during the study of drought tolerant maize for Africa (DTMA) and water efficient maize for Africa (WEMA) populations. A number of factors, including differences in the C_0 population size and number of markers used in selection

Table 3 : Analysis of variance for phenotypic stability of maize testcrosses involving DH lines from C_1, C_2 and C_3 of MPS 1 and MPS 2 populations evaluated under heat stress, optimal and early spring at Bheemarayanagudi and Raichur during 2018 and 2019.

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.

might have contributed for such differences.

The genetic gain for grain yield in MPS 1 population has reached up to C_2 which later declined in C_3 at across locations and situations (Table 4). This may be due to the fact that the top performing families were used to constitute cycles rather than the randomly selected families. The other reason, the DH lines which were produced from the initial source population may be random and the DH lines were used in the present investigation are from DH pipeline, so over the generations the genetic value may be decreased. Similar findings of inconsistency in genetic gains were also reported by Das *et al.* (2020), where they found linear gains from C_1 to C_3 in MYS-1 under drought stress, whereas under waterlogging stress MYS 1 showed gain from C_1 to C_2 and nominal loss between C_2 and C_3. It was evident from the data (Table 4) that under heat stress, early spring and optimal conditions, the gains in grain yield over selection cycles across location was consistent in MPS 1 but in MPS 2 gain was not consistent but showed differential response. Hosamani *et al.* (2020) also reported maximum gain for grain yield in population testcrosses of MPS 1 as compared to MPS 2 under heat stress and inferred that it could be due to the difference in population size and level of inbreeding between the two heterotic groups under heat stress condition.

The marginal positive gain of 29 kg ha^{-1} cycle^{-1} and 58 kg ha^{-1} cycle^{-1} under early spring and optimal condition, respectively was registered in MPS 2 population (Table 4); this may be explained by the fact that apart from stress tolerant trait donors, elite high yielding lines were also involved in

Table 4 : Mean grain yield (t ha^{-1}) of maize testcrosses involving DH lines from C_1, C_2 and C_3 of MPS 1 and MPS 2 populations evaluated under heat stress, optimal and early spring at Bheemarayanagudi (B'gudi) and Raichur during 2018 and 2019.

MPS 1 (Heterotic group A)																
	Heat stress				**Early spring**				**Optimal**				**Across situations mean**	**Genetic gain (%)**	**Across heatstress mean**	**Genetic gain (%)**
	B'gudi	**Raichur**	**Mean**	**Gain (%)**	**B'gudi**	**Raichur**	**Mean**	**Gain (%)**	**B'gudi**	**Raichur**	**Mean**	**Gain (%)**				
C_1	4.135	5.676	4.905	9.30	4.138	4.850	4.494	1.47	7.608	**8.854**	8.231	1.48	5.877	3.65	4.700	5.56
C_2	**5.008**	**5.716**	**5.362**	–23.15	**4.270**	**4.850**	**4.560**	–4.59	**7.861**	8.845	**8.353**	–10.96	**6.091**	–12.95	**4.961**	–14.62
C_3	2.726	5.515	4.121	–16.00	4.010	4.691	4.351	–3.19	6.977	7.897	7.437	–9.65	5.303	–9.77	4.236	–9.87
Mean	3.956	5.636	4.796		4.139	4.797	4.468		7.482	8.532	8.007		5.757		4.632	
Average of checks	3.245	5.020	4.132	29.75	3.948	4.686	4.317	5.63	7.941	7.306	7.624	9.56	5.358	13.70	4.225	17.43
Average gain per cycle	–0.705	–0.081	–0.393		–0.064	–0.079	–0.072		–0.316	–0.479	–0.397		–0.287		–0.232	
MPS 2 (Heterotic group B)																
	Heat stress				**Early spring**				**Optimal**				**Across situations mean**	**Genetic gain (%)**	**Across heatstress mean**	**Genetic gain (%)**
	B'gudi	**Raichur**	**Mean**	**Gain (%)**	**B'gudi**	**Raichur**	**Mean**	**Gain (%)**	**B'gudi**	**Raichur**	**Mean**	**Gain (%)**				
C_1	**5.957**	5.736	**5.847**	–16.11	4.096	4.672	4.384	4.11	**7.351**	8.272	7.811	0.99	**6.014**	–3.79	**5.115**	–7.44
C_2	4.063	**5.747**	4.905	–9.40	**4.110**	**5.019**	**4.564**	–2.73	7.277	8.499	7.888	0.50	5.786	–3.15	4.734	–6.19
C_3	3.229	5.658	4.443	–24.00	4.054	4.825	4.440	1.27	7.208	**8.648**	**7.928**	1.50	5.604	–6.82	4.441	–13.17
Mean	4.416	5.714	5.065		4.086	4.839	4.463		7.279	8.473	7.876		5.801		4.764	
Average of checks	3.245	5.020	4.132	41.48	3.948	4.686	4.317	5.73	7.941	7.306	7.624	3.99	5.358	12.25	4.225	21.08
Average gain per cycle	–1.364	–0.039	–0.702		–0.021	0.076	0.029		–0.072	0.188	0.058		–0.205		–0.337	

Note: % gain was calculated as, from C_1 to C_2, C_2 to C_3, C_1 to C_3 and average of checks to best cycle.

constituting base populations and the constitution of first cycle (C_1) was based on selecting phenotypically superior testcross progenies across heat stress and optimal conditions. These findings suggest that, the stress tolerance may not necessarily be associated with yield penalties under early spring and optimal condition. Similarly, Semagn *et al*. (2015) reported an average gain of 184 kg ha^{-1} cycle^{-1} under well-watered conditions in bi– parental maize populations. Beyene *et al*. (2016) reported an average gain of 105 kg ha^{-1} year^{-1} under well– watered condition. Das *et al*. (2020) also found the nominal positive gains in MYS-1 (32 kg ha^{-1} cycle^{-1}) and MYS-2 (46 kg ha^{-1} cycle^{-1}) populations under optimal moisture conditions.

Genetic gain in mean plant height

The plant height is the important trait indicating the heats stress tolerance; because heat stress reduces the inter-nodal elongation, which results in reduced plant height and also ear height in the same proportion (Zaidi *et al*., 2016). Under heat stress and optimal situation (Table 5), the C_2 testcrosses in MPS 1 and MPS 2 population produced higher plant height compared to other cycles across locations. Thus both the populations registered positive response to selection by producing higher plant height at C_2 compared to C_1. In case of early spring situation, the C_2 testcrosses of MPS 2 produced higher plant height than other cycles, whereas the MPS 1 population did not show increased plant height over cycles across locations. In the present study, the effect of high temperature on plant height of maize can be clearly seen under across situations (Table 5). It was evident that over all reduced plant height (122.27 cm) under heat stress and early spring situation (128.84 cm) was observed as compared to optimal situation (191.35 cm). The response to selection in cycles of MPS 1 and MPS 2 populations under heat stress condition was consistent across situations. The genetic gain across situations in MPS 1 and MPS 2 populations has reached up to C_2 which later declined in C_3. Hosamani *et al*. (2020) reported increased plant height from base population to $F_{2\text{-}3}$ both in MPS 1 and MPS 2, respectively thus showed positive response to selection under heat stress condition. Zhang *et al*. (2017) reported that, the plant and ear heights for the two latest GS cycles were ~6 to 10 cm higher than the plant and ear heights of the maize plants for the two earlier GS cycles of multi parent populations.

Genetic gain in mean number of kernels per cob

The kernel number per cob is also an indicator of seed set under high temperature, because under heat stress condition kernel numbers tend to decrease due to reduced pollen viability, pollen desiccation, pollination failure, kernel abortion, shortened grain filling period *etc*., which ultimately limit the kernel number and weight (Cicchino *et al*., 2010 and Waqas *et al*., 2021). It is evident from data (Table 6) that, the MPS 2 population under early spring and optimal condition exhibited 0.53% and 0.87% increased number of kernels per cob from C_1 to C_2 across locations indicating positive response to selection, but the number of kernels per cob got decreased from C_2 to C_3 and C_1 to C_3 under both situations. Whereas, MPS 2 population under heat stress condition and at across all situations, both MPS 1 and MPS 2 population recorded gradually decreased number of kernels per cob from C_1 to C_2, C_2 to C_3 indicating negative response to selection. The regression analysis of selection cycles also showed decreased number of kernels per selection cycle across locations and situations. Thus, the MPS 2 population responded comparatively better to rapid cycle genomic selection under early spring and optimal conditions. Hosamani (2019) reported positive response to selection or genetic gain in MPS 1 and MPS 2 populations for number of kernels per cob across cycles *i.e.*, gain was observed up to C_3 cycle across two locations under optimal conditions and heat stress.

Genetic gain in mean anthesis to silking interval (ASI)

The minimum ASI is desirable for any commercial maize breeding indicating good synchrony between tasseling and silking. The data (Table 7) evidently showed that, among two populations, increased gains from C_1 to C_2 was recorded in MPS 2 population considering across locations and situations, thereafter ASI got slightly increased from C_2 to C_3. In contrast, the MPS 1 population under all the environment situations did not exhibit significant change or improvement over cycles, indicating that the DH testcrosses were at similar phenological stage. Generally, the increase in the ASI is undesirable, which was found in the present study from C_1 to C_3 in MPS 1, this was may be due to phenotypic plasticity under stress situation. It is also observed that both the populations across situations maintained good synchrony (< 3.0 days) between male and female flowering except in MPS 1 and MPS 2 at Bheemarayanagudi under early spring condition. These findings are in agreement with Das *et al*. (2020), who reported significant decrease in ASI from C_1 to C_3 in MYS-1 population under drought stress and waterlogging condition and found MYS-2 population has responded comparatively better to RCGS. Hosamani *et al*. (2020) also found improvement in ASI from base population to $F_{2\text{-}3}$ families under heat stress condition.

Table 5 : Mean plant height (cm) of maize testcrosses involving DH lines from C_1, C_2 and C_3 of MPS 1 and MPS 2 populations evaluated under heat stress, optimal and early spring at Bheemarayanagudi (B'gudi) and Raichur during 2018 and 2019.

	Heat stress				Early spring				Optimal				Across situations mean	Genetic gain (%)	Across heatstress mean	Genetic gain (%)
	B'gudi	Raichur	Mean	Gain (%)	B'gudi	Raichur	Mean	Gain (%)	B'gudi	Raichur	Mean	Gain (%)				
MPS 1 (Heterotic group A)																
C_1	118.76	126.78	122.77	0.43	**123.66**	137.08	**130.37**	–0.12	**218.27**	170.24	194.26	0.25	149.13	0.19	126.57	0.15
C_2	**119.45**	**127.15**	**123.30**	–2.65	122.73	137.70	130.21	–2.88	217.42	**172.06**	**194.74**	–3.32	**149.42**	–3.01	**126.76**	–2.77
C_3	113.59	126.49	120.04	–2.22	115.18	**137.76**	126.47	–2.99	214.04	162.52	188.28	–3.08	144.93	–2.82	123.25	–2.62
Mean	117.27	126.81	122.04		120.52	137.51	129.02		216.58	168.27	192.43		147.83		125.53	
Average of checks	119.40	130.74	125.07	–1.41	126.57	138.02	132.30	–1.46	226.92	170.94	198.93	–2.11	152.10	–1.76	128.69	–1.50
Average gain per cycle	–2.58	–0.15	–1.37		–4.24	0.34	–1.95		–2.12	–3.86	–2.99		–2.10		–1.66	
MPS 2 (Heterotic group B)																
	Heat stress				**Early spring**				**Optimal**				**Across situations mean**	**Genetic gain (%)**	**Across heatstress mean**	**Genetic gain (%)**
	B'gudi	**Raichur**	**Mean**	**Gain (%)**	**B'gudi**	**Raichur**	**Mean**	**Gain (%)**	**B'gudi**	**Raichur**	**Mean**	**Gain (%)**				
C_1	117.47	126.00	121.73	1.50	117.69	137.37	127.53	2.28	211.68	165.13	188.41	2.91	145.89	2.33	124.63	1.90
C_2	**118.52**	128.59	**123.56**	–1.06	**122.24**	138.64	**130.44**	–1.86	**219.38**	168.39	**193.88**	–2.77	**149.29**	–2.03	**127.00**	–1.47
C_3	114.81	**129.69**	122.25	0.42	116.88	**139.15**	128.02	0.38	208.57	**168.45**	188.51	0.05	146.26	0.25	125.13	0.40
Mean	116.93	128.09	122.51		118.94	138.39	128.66		213.21	167.33	190.27		147.15		125.59	
Average of checks	119.40	130.74	125.07	–1.21	126.57	138.02	132.30	–1.40	226.92	170.94	198.93	–2.54	152.10	–1.85	128.69	–1.31
Average gain per cycle	–1.33	1.84	0.26		–0.40	0.89	0.24		–1.56	1.66	0.05		0.18		0.25	

Note: % gain was calculated as, from C_1 to C_2, C_2 to C_3, C_1 to C_3 and average of checks to best cycle.

Table 6 : Mean number of kernels per cob of maize testcrosses involving DH lines from C_1, C_2 and C_3 of MPS 1 and MPS 2 populations evaluated under heat stress, optimal and early spring at Bheemarayanagudi (B'gudi) and Raichur during 2018 and 2019.

MPS 1 (Heterotic group A)																
	Heat stress				Early spring				Optimal				Across situations mean	Genetic gain (%)	Across heatstress mean	Genetic gain (%)
	B'gudi	Raichur	Mean	Gain (%)	B'gudi	Raichur	Mean	Gain (%)	B'gudi	Raichur	Mean	Gain (%)				
C_1	267.86	**353.79**	**310.83**	–0.45	330.96	**390.85**	**360.90**	–0.09	**397.83**	**379.83**	**388.83**	–0.10	**353.52**	–0.20	**335.86**	–0.26
C_2	**292.52**	326.31	309.41	–16.37	**332.63**	388.52	360.58	–1.92	397.82	379.05	388.44	–5.50	352.81	–7.46	335.00	–8.60
C_3	197.12	320.38	258.75	–16.75	323.73	383.55	353.64	–2.01	368.93	365.19	367.06	–5.60	326.48	–7.65	306.19	–8.83
Mean	252.50	333.50	293.00		329.11	387.64	358.37		388.19	374.69	381.44		344.27		325.69	
Average of checks	217.38	308.22	262.80	18.27	321.47	377.95	349.71	3.20	387.01	343.09	365.05	6.51	325.85	8.49	306.26	9.67
Average gain per cycle	–35.37	–16.71	–26.04		–3.61	–3.65	–3.63		–14.45	–7.32	–10.89		–13.52		–14.84	
MPS 2 (Heterotic group B)																
	Heat stress				Early spring				Optimal				Across situations mean	Genetic gain (%)	Across heatstress mean	Genetic gain (%)
	B'gudi	Raichur	Mean	Gain (%)	B'gudi	Raichur	Mean	Gain (%)	B'gudi	Raichur	Mean	Gain (%)				
C_1	**330.61**	349.25	**339.93**	–10.64	325.02	385.73	355.37	0.53	386.50	369.04	377.77	0.87	**357.69**	–2.89	**347.65**	–4.93
C_2	254.81	**352.69**	303.75	–7.77	**326.06**	**388.49**	**357.27**	–0.90	**387.78**	**374.33**	**381.06**	–2.28	347.36	–3.41	330.51	–4.06
C_3	211.58	348.74	280.16	–17.58	320.00	388.08	354.04	–0.37	370.86	373.86	372.36	–1.43	335.52	–6.20	317.10	–8.79
Mean	265.67	350.23	307.95		323.69	387.43	355.56		381.71	372.41	377.06		346.86		331.76	
Average of checks	217.38	308.22	262.80	29.35	321.47	377.95	349.71	2.16	387.01	343.09	365.05	4.38	325.85	9.77	306.26	13.52
Average gain per cycle	–59.52	–0.26	–29.89		–2.51	1.18	–0.67		–7.82	2.41	–2.71		–11.09		–15.28	

Note: % gain was calculated as, from C_1 to C_2, C_2 to C_3, C_1 to C_3 and average of checks to best cycle.

Table 7 : Mean anthesis to silking interval of maize testcrosses involving DH lines from C_1, C_2 and C_3 of MPS 1 and MPS 2 populations evaluated under heat stress, optimal and early spring at Bheemarayanagudi (B'gudi) and Raichur during 2018 and 2019.

MPS 1 (Heterotic group A)																
	Heat stress				Early spring				Optimal				Across situations mean	Genetic gain (%)	Across heatstress mean	Genetic gain (%)
	B'gudi	Raichur	Mean	Gain (%)	B'gudi	Raichur	Mean	Gain (%)	B'gudi	Raichur	Mean	Gain (%)				
C_1	**2.39**	**1.44**	**1.92**	11.94	3.12	**2.20**	**2.66**	0.77	**1.91**	**2.08**	**2.00**	6.84	**2.19**	5.87	**2.29**	5.45
C_2	2.57	1.72	2.15	6.14	3.12	2.24	2.68	1.04	2.03	2.23	2.13	18.28	2.32	7.90	2.41	3.31
C_3	2.81	1.74	2.28	18.81	3.12	2.29	2.71	1.82	2.45	2.60	2.52	26.37	2.50	14.24	2.49	8.94
Mean	2.59	1.64	2.11		3.12	2.24	2.68		2.13	2.31	2.22		2.34		2.40	
Average of checks	2.81	1.84	2.32	–17.52	3.12	2.25	2.68	–0.96	2.14	2.28	2.21	–9.68	2.41	–8.96	2.50	–8.64
Average gain per cycle	0.21	0.15	0.18		3.12	0.05	0.02		0.27	0.26	0.26		0.16		0.10	
MPS 2 (Heterotic group B)																
	Heat stress				Early spring				Optimal				Across situations mean	Genetic gain (%)	Across heatstress mean	Genetic gain (%)
	B'gudi	Raichur	Mean	Gain (%)	B'gudi	Raichur	Mean	Gain (%)	B'gudi	Raichur	Mean	Gain (%)				
C_1	2.61	1.48	2.05	–4.34	3.12	2.21	2.66	–0.70	2.17	2.43	2.30	–8.50	2.34	–4.32	2.36	–2.28
C_2	**2.58**	1.33	**1.96**	4.95	3.12	**2.17**	**2.65**	0.31	**1.98**	2.23	**2.10**	0.07	**2.24**	1.59	**2.30**	2.28
C_3	2.80	**1.31**	2.06	0.39	3.12	2.19	2.65	–0.40	2.10	**2.12**	2.11	–8.44	2.27	–2.81	2.35	–0.06
Mean	2.66	1.38	2.02		3.12	2.19	2.65		2.08	2.26	2.17		2.28		2.34	
Average of checks	2.81	1.83	2.32	–15.74	3.12	2.25	2.68	–1.39	2.14	2.28	2.21	–4.75	2.41	–7.04	2.50	–8.05
Average gain per cycle	0.09	–0.08	0.00		3.12	–0.01	–0.01		–0.04	–0.16	–0.10		–0.03		–0.00	

Note: Gain (%) was calculated as, from C_1 to C_2, C_2 to C_3, C_1 to C_3 and average of checks to best cycle.

Table 8 : Mean leaf firing (%) and tassel blast (%) of maize testcrosses involving DH lines from C_1, C_2 and C_3 of MPS 1 and MPS 2 populations evaluated under heat stress at Bheemarayanagudi (B'gudi) and Raichur during summer 2018.

Leaf firing (%)												
	Heat stress											
	MPS 1 (Heterotic group A)						**MPS 2 (Heterotic group B)**					
Cycle	**B'gudi**	**Gain (%)**	**Raichur**	**Gain (%)**	**Across**	**Gain (%)**	**B'gudi**	**Gain (%)**	**Raichur**	**Gain (%)**	**Across**	**Gain (%)**
C_1	1.18	“5.40	0.93	0.88	1.05	“2.64	0.92	1.15	**0.89**	3.17	0.91	2.14
C_2	1.12	“21.21	0.94	“3.33	1.03	“13.06	0.93	“9.02	0.92	“2.07	0.93	“5.56
C_3	**0.88**	“25.47	**0.90**	“2.48	**0.89**	“15.35	**0.85**	“7.97	0.90	1.04	**0.88**	“3.54
Cycle Mean	1.06		0.92		0.99		0.90		0.91		0.90	
Average of checks	0.92	“4.63	0.92	“1.77	0.92	“3.20	0.92	“7.86	0.92	“2.81	0.92	“4.84
Average gain per cycle	“0.15		“0.01		“0.08		“0.04		0.00		“0.02	
Tassel blast (%)												
Heat stress												
	MPS 1 (Heterotic group A)						**MPS 2 (Heterotic group B)**					
Cycle	**B'gudi**	**Gain (%)**	**Raichur**	**Gain (%)**	**Across**	**Gain (%)**	**B'gudi**	**Gain (%)**	**Raichur**	**Gain (%)**	**Across**	**Gain (%)**
C_1	2.87	14.45	0.74	0.00	1.81	11.49	**1.63**	48.97	0.74	0.00	**1.19**	33.66
C_2	3.29	“42.42	0.74	0.00	2.01	“34.62	2.43	“18.48	0.74	0.00	1.58	“14.16
C_3	**1.89**	“34.10	**0.74**	0.00	**1.32**	“27.11	1.98	21.44	**0.74**	0.00	1.36	14.73
Cycle Mean	2.68		0.74		1.71		2.01		0.74		1.38	
Average of checks	1.15	65.24	0.74	0.00	0.94	39.60	1.15	42.26	0.74	0.00	0.94	25.65
Average gain per cycle	“0.49		0.00		“0.24		0.17		0.00		0.09	

Note: Gain (%) was calculated as, from C_1 to C_2, C_2 to C_3, C_1 to C_3 and average of checks to best cycle. Tassel blast was not observed during optimal and early spring condition.

In addition to this, under heat stress condition (Table 7), the MPS 2 showed improvement in ASI from C_1 to C_2 at Bheemarayanagudi and across locations; but at Raichur C_3 registered highest ASI of 1.31 days under heat stress which was also lesser than checks. It clearly demonstrated that genomic selection tended to reduce ASI in MPS 2 population under heat stress. Alam *et al.* (2017) reported that, an ASI of 2 to 4 days and pollen shedding duration of 2 to 4 days were found advantageous to grain yield under heat stress. Stigma receptivity was less affected under heat stress when compared to pollen viability, yet stigma initiation was delayed under heat stress, which resulted in prolonged ASI. In the present study, the marginal improvement of ASI in MPS 2 population indicates that, it is important to maintain a selection pressure for reduced ASI to ensure as it remains below 3 days under stress conditions.

Genetic gain in mean leaf firing and tassel blast

Leaf firing (%) and Tassel blast (%) are the typical symptoms of heat stress and these two symptoms often appear together. The genotypes with none of these symptoms or at the most reduced incidence under heat stress condition is desirable for commercial maize breeding (Zaidi *et al.*, 2016). In the present study, the incidence of these two were observed only under heat stress condition and were not observed under early spring and optimal conditions. The mean leaf firing and tassel blast (Table 8) across locations showed that, the MPS 1 population exhibited reduced incidence of these symptoms from C_1 to C_2, C_2 to C_3 and C_1 to C_3. Whereas, the cycles of MPS 2 population across locations registered differential response to tassel blast, *i.e.*, reduced leaf firing incidence from C_2 to C_3 and C_1 to C_3 and the cycles of MPS 2 population documented increased incidence of tassel blast from C_1 to C_2 and decreased incidence of tassel blast from C_2 to C_3. In the present study under heat stress condition, the reduction in per cent leaf firing and tassel blast incidence was desirable and that improvement could be considered as genetic gain. The MPS 1 and 2 populations across locations exhibited positive response to genetic gain across cycles for leaf firing; while for tassel blast, the MPS 1 population across locations exhibited improved genetic gain across cycles when compared to MPS 2 population. It is to note that, the integration of rapid cycle genomic selection in MPS 1 and 2 populations has simultaneously improved the leaf firing and tassel blast traits along with the increased genetic gain for grain yield. Hosamani (2019) also reported the reduced incidence of leaf firing from C_2 to C_3 in MPS 1 and MPS 2 in population testcrosses involving complementary tester across two locations under heat stress condition.

Findings of the study are the first of its kind to report rapid cycle genomic selection for heat stress tolerance in maize using double haploids derived from improved cycles of multi-parental synthetic populations 1 and 2. From the present study it is to infer that, the two populations, MPS 1 and MPS 2 showed a differential response to rapid cycle genomic selection *i.e.*, consistent increase from C_1 to C_2 and then yield got declined in C_3 for the traits *viz.*, grain yield, anthesis to silking interval, plant height, number of kernels per cob and 100 grain weight. Particularly for grain yield, the MPS 1 population responded better to genomic selection compared to MPS 2 population. The differential response of the two populations to RCGS suggested that the strength of lines/trait donors involved in constituting the base population plays a key role in the genetic gain with genomic selection. Therefore, care should be taken during initial stage of evaluation after constituting base population and move forward in genomic selection to save on genotyping costs. Besides, it is also evident that the RCGS has simultaneously improved the anthesis to silking interval, leaf firing and tassel blast traits along with increased grain yield in both populations.

Acknowledgement

Authors are very much thankful to USAID for financial support through “Heat Tolerant Maize for Asia” through public private partnership (HTMA-II) project.

Authors’ contributions

Conceptualization of research (PHK, PHZ, VMT); Designing of the experiments (NS, PHK, PHZ); Contribution of experimental materials (PHZ, VMT); Execution of field/lab experiments and data collection (NS, PHK); Investigation and suggestions (NS, AB, AP, SHC, DMM); Analysis of data and interpretation (NS, VMT, PHK); Preparation of the manuscript (NS, PHK). All authors have read and agree to the published version of the manuscript.

Conflict of interest: None

Source of Support: Nil

Data Availability Statement: All data generated during study are included in this article.

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