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STABILITY OF MAIZE HYBRIDS DEVELOPED THROUGH INTEGRATION OF RAPID CYCLE GENOMIC SELECTION AND DOUBLED HAPLOID TECHNOLOGY

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ABSTRACT

Maize is highly sensitive to environmental conditions, especially heat stress which leads to severe yield loss. For ever changing climate, it is very much necessary to develop cultivars with improved heat tolerance using appropriate genetic approaches. Hence, the present study was designed to assess the stability of 111 doubled haploid testcross hybrids which were derived from C_1, C_2 and C_3 cycles of multi-parental synthetic (MPS) population 1 and 2, developed through the integration of genomic selection and doubled haploid technology. The experiment was laid out in alpha lattice design across two locations Bheemarayanagudi and Raichur in Karnataka during 2018 and 2019 under heat stress, early spring and optimal growing conditions. The approach of Eberhart and Russell (1966) was used to assess the stability of hybrids for major characters. Among the 111 hybrids, the testcross CML451/(MPS-1-C2GS)-DH7 had best mean value for grain yield (5.476 t ha^{-1}), β_i nearer to unity (0.905) and non-significant S^2d_i (-0.295), thus identified as stable over studied environmental situations. This hybrid needs to be tested extensively at different locations under different situations for its suitability for commercialization besides using in new hybrid combinations. Similarly, one testcross CML451/(MPS-2-C1)-DH8 was found stable for plant height and three testcrosses *viz.*, CML451/(MPS-1-C2GS)-DH30, CML451/(MPS-2-C1)-DH73 and CML451/(MPS-2-C2GS)-DH65 were identified as stable for number of kernels per cob. Therefore, these DH's could be incorporated in breeding stocks for further use.

***Key words*:** Maize, heat stress, rapid cycle genomic selection, doubled haploid, stability.

Introduction

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 (Alam *et al.*, 2017). 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 (FAO, 2015). Current food production rate combined with population growth and predicted impacts of climate change will not suffice to meet future food demand. Improving crop productivity and livelihoods of smallholders under increasing climate variability will require a multi-disciplinary approach towards crop genetic improvement (Hansen *et al*., 2019). 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 (Prasanna *et al*., 2021).

Maize productivity is very high under optimal environmental and crop management conditions. However, maize yields in the tropical rain fed environments are now increasingly vulnerable to various climate induced stresses, especially drought, heat, waterlogging, salinity, cold, diseases and insect pests which often come in combinations to severely impact maize production (Prasanna *et al*., 2021). Further, the production of maize during post rainy spring season is emerging as a potential segment for expanding the maize production niche in South Asia, but the production capacity is often challenged due to heat stress (Vinayan *et al*., 2020). Most of the maize area in India is under rain-fed conditions majorly grown by the small holder and resource poor farmers; which is also vulnerable to extreme weather events, including drought and high temperatures. This adds further challenges to the existing problems of millions of maize-dependent smallholding farmers in the tropics including India and it undermines the progress being made to improve the food security and marginal incomes.

Heat stress is also becoming major constraint to maize production. The rise in temperature beyond threshold level can cause irreversible damage to crop growth and yield. Temperature above 35°C for a longer period is considered to be unfavourable for maize and above 40°C may cause irreversible damage and detrimental effects to the crop. The impacts of heat stress can be at any stage of the crop; 14 to 17% yield penalty during tasseling stage, 13% at silking stage, 10 to 45% at pre and post flowering, 31% yield reduction at grain filling stage was reported in different maize growing regions (Waqas *et al*., 2021). These adverse effects of heat stress can be mitigated by developing crop plants with improved thermo tolerance using various genetic approaches. So, there is a need to expedite the development of stable and adaptable variety/ hybrid for climate resilience by utilizing modern breeding tools like genomic selection and double haploid (DH) technology in a maize breeding programme.

There is limited information available in the literature on the level of genotype × environment interaction and stability of maize DH hybrids improved for heat stress tolerance in tropical maize especially in India. However, Jodage *et al*., (2018) and Gazala *et al*., (2019) studied the combining ability of maize inbred lines under heat stress conditions. Geetha *et al*., (2019) assessed the general combining ability of maize DH lines, specific combining ability and heterotic potential of DH hybrids. Patil *et al*., (2022) assessed the general combining ability and specific combining ability among the selected superior parental lines and DH testcrosses for maize grain yield under heat stress. Further, stability analysis of newly developed hybrids under heat stress and optimal conditions was also carried out by Archana *et al*., (2018), Divya *et al*., (2019) and Pavani *et al*., (2019). The studies on identifying the major environmental variables contributing to differential performance of tropical maize hybrids and identification of stable hybrids across heat stress environments in South Asia (Vinayan *et al*., 2019 and Vinayan *et al*., 2020) were also emphasizes the need of heat stress resilient cultivars. Hosamani (2019), Hosamani *et al*., (2020) and Swamy *et al*., (2024) reported genetic gain across testcrosses of different cycles of multi-parental synthetic populations (MPS 1 and MPS 2) improved through rapid cycle genomic selection (RCGS) for various traits under heat stress and optimal conditions. In the present study, we are reporting the stability of 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, Bheemarayanagudi 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. Bheemarayanagudi and Raichur come under north-eastern dry zone of Karnataka, India. The weather data during crop growth period indicated that most of the cropping period during summer 2018 was under heat stress. Thus, the combination of high temperature ($T_{max.}$ > 35 °C and $T_{min.}$ e" 21 °C) and relative humidity (< 50%) ensured the proper evaluation of trial. Therefore, the warm and dry humid climatic conditions

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.

MPS 1 population (HGA)	
Sl. No.	**Pedigree**
1	CML451/(MPS-1-C1)-DH1
2	CML451/(MPS-1-C1)-DH3
3	CML451/(MPS-1-C1)-DH9
4	CML451/(MPS-1-C1)-DH11
5	CML451/(MPS-1-C1)-DH12
6	CML451/(MPS-1-C1)-DH24
7	CML451/(MPS-1-C1)-DH26
8	CML451/(MPS-1-C1)-DH27
9	CML451/(MPS-1-C1)-DH28
10	CML451/(MPS-1-C1)-DH30
11	CML451/(MPS-1-C1)-DH34
12	CML451/(MPS-1-C1)-DH36
13	CML451/(MPS-1-C1)-DH38
14	CML451/(MPS-1-C1)-DH40
15	CML451/(MPS-1-C1)-DH41
16	CML451/(MPS-1-C1)-DH45
17	CML451/(MPS-1-C1)-DH46
18	CML451/(MPS-1-C1)-DH47
19	CML451/(MPS-1-C1)-DH49
20	CML451/(MPS-1-C1)-DH50
21	CML451/(MPS-1-C1)-DH55
22	CML451/(MPS-1-C1)-DH58
23	CML451/(MPS-1-C1)-DH59
24	CML451/(MPS-1-C1)-DH60
25	CML451/(MPS-1-C1)-DH61
26	CML451/(MPS-1-C1)-DH64
27	CML451/(MPS-1-C1)-DH65
28	CML451/(MPS-1-C1)-DH66
29	CML451/(MPS-1-C1)-DH68
30	CML451/(MPS-1-C1)-DH69
31	CML451/(MPS-1-C1)-DH72
32	CML451/(MPS-1-C1)-DH74
33	CML451/(MPS-1-C1)-DH75
34	CML451/(MPS-1-C2GS)-DH2
35	CML451/(MPS-1-C2GS)-DH5
36	CML451/(MPS-1-C2GS)-DH7
37	CML451/(MPS-1-C2GS)-DH12
38	CML451/(MPS-1-C2GS)-DH16
39	CML451/(MPS-1-C2GS)-DH21
40	CML451/(MPS-1-C2GS)-DH22
41	CML451/(MPS-1-C2GS)-DH25
42	CML451/(MPS-1-C2GS)-DH29
43	CML451/(MPS-1-C2GS)-DH30
44	CML451/(MPS-1-C2GS)-DH33
45	CML451/(MPS-1-C2GS)-DH37
46	CML451/(MPS-1-C2GS)-DH40
47	CML451/(MPS-1-C2GS)-DH42
48	CML451/(MPS-1-C2GS)-DH45
49	CML451/(MPS-1-C2GS)-DH56
50	CML451/(MPS-1-C2GS)-DH61
51	CML451/(MPS-1-C2GS)-DH64
52	CML451/(MPS-1-C2GS)-DH67
53	CML451/(MPS-1-C2GS)-DH70
54	CML451/(MPS-1-C3GS)-DH2
55	CML451/(MPS-1-C3GS)-DH3
56	CML451/(MPS-1-C3GS)-DH5
57	CML451/(MPS-1-C3GS)-DH8
58	CML451/(MPS-1-C3GS)-DH15
59	CML451/(MPS-1-C3GS)-DH18
60	CML451/(MPS-1-C3GS)-DH19
61	CML451/(MPS-1-C3GS)-DH29
62	CML451/(MPS-1-C3GS)-DH30
63	CML451/(MPS-1-C3GS)-DH31
64	CML451/(MPS-1-C3GS)-DH34
65	CML451/(MPS-1-C3GS)-DH38
66	CML451/(MPS-1-C3GS)-DH49
67	CML451/(MPS-1-C3GS)-DH64
MPS 2 population (HGB)	
Sl. No.	**Pedigree**
68	CML451/(MPS-2-C1)-DH8
69	CML451/(MPS-2-C1)-DH14
70	CML451/(MPS-2-C1)-DH15
71	CML451/(MPS-2-C1)-DH16
72	CML451/(MPS-2-C1)-DH29
73	CML451/(MPS-2-C1)-DH36
74	CML451/(MPS-2-C1)-DH37
75	CML451/(MPS-2-C1)-DH40
76	CML451/(MPS-2-C1)-DH44
77	CML451/(MPS-2-C1)-DH47
78	CML451/(MPS-2-C1)-DH53
79	CML451/(MPS-2-C1)-DH56
80	CML451/(MPS-2-C1)-DH70
81	CML451/(MPS-2-C1)-DH73
82	CML451/(MPS-2-C2GS)-DH1
83	CML451/(MPS-2-C2GS)-DH2
84	CML451/(MPS-2-C2GS)-DH3
85	CML451/(MPS-2-C2GS)-DH9
86	CML451/(MPS-2-C2GS)-DH15
87	CML451/(MPS-2-C2GS)-DH19
88	CML451/(MPS-2-C2GS)-DH20
89	CML451/(MPS-2-C2GS)-DH28
90	CML451/(MPS-2-C2GS)-DH31
91	CML451/(MPS-2-C2GS)-DH42
92	CML451/(MPS-2-C2GS)-DH48
93	CML451/(MPS-2-C2GS)-DH49
94	CML451/(MPS-2-C2GS)-DH57
95	CML451/(MPS-2-C2GS)-DH60

Continue 2...

96	CML451/(MPS-2-C2GS)-DH65
97	CML451/(MPS-2-C2GS)-DH74
98	CML451/(MPS-2-C2GS)-DH75
99	CML451/(MPS-2-C3GS)-DH1
100	CML451/(MPS-2-C3GS)-DH10
101	CML451/(MPS-2-C3GS)-DH11
102	CML451/(MPS-2-C3GS)-DH13
103	CML451/(MPS-2-C3GS)-DH22
104	CML451/(MPS-2-C3GS)-DH26
105	CML451/(MPS-2-C3GS)-DH30
106	CML451/(MPS-2-C3GS)-DH35
107	CML451/(MPS-2-C3GS)-DH39
108	CML451/(MPS-2-C3GS)-DH45
109	CML451/(MPS-2-C3GS)-DH50
110	CML451/(MPS-2-C3GS)-DH62
111	CML451/(MPS-2-C3GS)-DH68

Summary of DH testcrosses involving different cycles of MPS populations

Sl. 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

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, 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 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. 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 to identify stable hybrids 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 (Table 1). 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 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

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 later stages 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 later stages of crop growth under irrigation	21.03	34.63	23.50	48.00

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 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 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 package. To analyze the stability parameters of major characters and for each genotype, the approach of Eberhart and Russell (1966) was used and the basic model involves the estimation of three parameters which are defined by a mathematical formula as given below.

$$Y_{ij} = \mu_i + \beta_i I_j + S_{ij}$$

Where,

Y_{ij} = Mean of the ith genotype at the jth environment

μ_i = Mean of ith genotype over all environments

β_i = The regression coefficient that measures the response of ith genotype to varying environments

S_{ij} = The deviation from regression of the ith genotype at the jth environment and

I_j = The environmental index obtained by subtracting the grand mean from the mean of all genotype at the jth environment

As per this model, an ideal stable genotype is one which has high mean value than population mean as the character has economic importance, a regression coefficient equals to unity ($\beta_i = 1$) and a minimum mean square deviation from linear regression statistically equal

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)	No. 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.

to zero (S^2d_i = 0). When the deviations are non-significant, the conclusions may be drawn by joint consideration of mean and regression values as per Finlay and Wilkinson (1963) and Eberhart and Russell (1966); the genotypes can be classified as average (β_i = 1 and with high mean), below average (β_i >1 and with high mean) and above average (β_i <1 and with high mean) stability. To measure the stability of individual genotype and adaptability to varying and harsh environments, the software GEA-R version 4.1 (Genotype × Environment Analysis with R) from CIMMYT repository was utilized (Angela *et al.*, 2015). For the analysis of stability parameters, only the data on heat stress and early spring condition were utilized.

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 condition at Bheemarayanagudi and Raichur during 2018 and 2019 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 which indicated the three imposed environments *viz.*, heat stress (summer), optimal (*kharif*) and early spring (late *rabi*) were different and the characters behaved differently in each season. The mean sum of squares due to location were non-significant all the traits show that, 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 cycle 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 differed significantly for grain yield and MPS 2 showed significant variation among genotypes for all the characters studied across two locations under heat stress condition. Archana *et al.*, (2018) also reported significant genotype and environment effects for grain yield and other traits in maize under heat stress. Pavani *et al.*, (2019) also found significant variations among the hybrids for all the traits across situations including heat stress.

Table 4: Stability parameters (Eberhart & Russell, 1966) for grain yield (t ha^{-1}) of selected testcross hybrids involving DH lines from C_1, C_2 and C_3 of MPS 1 and MPS 2 populations evaluated under heat stress and early spring at Bheemarayanagudi (B'gudi) and Raichur during 2018 and 2019.

S. no.	Pedigree	Performance under				General mean	S^2d_i	β_i	Performance under optimal condition	
		Heat stress		Early spring						
		E_1	E_2	E_3	E_4				B'gudi	Raichur
1	CML451/(MPS-1-C2GS)-DH7	4.809	5.756	4.327	4.966	5.476	-0.295	0.905	8.862	8.934
2	CML451/(MPS-1-C2GS)-DH56	3.640	4.935	3.941	4.563	4.240	-0.281	0.637	6.958	8.828
3	CML451/(MPS-2-C3GS)-DH50	2.302	5.941	3.956	4.668	4.570	-0.281	2.506	6.857	9.110
4	CML451/(MPS-1-C1)-DH45	3.192	5.766	4.066	4.703	4.044	0.007	1.257	7.170	7.847
5	CML451/(MPS-1-C1)-DH66	1.680	5.252	3.771	4.811	3.460	-0.134	1.732	7.175	8.859
6	CML451/(MPS-1-C2GS)-DH5	1.922	5.304	3.915	4.679	3.882	0.116	1.781	6.220	5.726
7	CML451/(MPS-1-C2GS)-DH37	2.639	5.673	3.957	5.137	4.740	0.141	2.047	7.144	8.173
8	CML451/(MPS-1-C3GS)-DH2	2.015	5.527	4.053	4.636	4.271	-0.080	1.886	6.403	7.857
9	CML451/(MPS-1-C3GS)-DH3	3.046	5.652	3.853	4.434	4.387	0.028	1.552	7.122	7.423
10	CML451/(MPS-1-C3GS)-DH8	1.542	5.546	3.848	4.562	3.944	-0.117	2.169	6.973	7.672
11	CML451/(MPS-1-C3GS)-DH15	1.226	5.300	3.860	4.394	3.761	-0.054	2.144	8.159	7.965
12	CML451/(MPS-1-C3GS)-DH19	1.675	5.938	4.025	4.569	4.450	-0.098	2.537	6.674	7.811
13	CML451/(MPS-1-C3GS)-DH29	1.922	5.600	4.096	4.897	4.541	0.265	2.097	6.573	7.986
14	CML451/(MPS-1-C3GS)-DH31	3.498	5.616	3.887	4.838	4.881	-0.001	1.495	6.883	8.153
15	CML451/(MPS-1-C3GS)-DH49	1.675	5.131	3.863	4.758	3.927	0.218	1.789	6.104	6.746
16	CML451/(MPS-1-C3GS)-DH64	1.881	5.051	3.764	4.787	3.932	0.126	1.717	7.996	7.180
17	CML451/(MPS-2-C2GS)-DH31	0.925	5.757	3.941	4.579	4.163	0.589	2.544	6.281	8.455
18	CML451/(MPS-2-C3GS)-DH11	3.312	6.380	3.743	5.137	5.301	0.367	2.876	8.151	7.726
19	CML451/(MPS-2-C3GS)-DH22	2.550	5.359	4.113	4.557	4.288	0.152	1.656	7.513	8.694
20	CML451/(MPS-2-C3GS)-DH26	1.952	5.569	3.831	4.193	3.732	-0.140	2.029	5.775	9.789
21	CML451/(MPS-2-C3GS)-DH39	3.398	5.528	3.936	4.291	4.383	0.060	1.599	7.531	8.724
22	CML451/(MPS-2-C3GS)-DH62	2.866	5.463	3.925	4.740	4.359	-0.075	1.639	6.268	9.877
Checks	900MG	3.066	4.838	3.738	4.370	4.014	-0.284	0.975	7.259	7.049
	DKC9108	2.080	3.999	3.733	4.474	3.533	0.658	0.852*	6.720	5.585
	NK 6240	3.548	5.261	3.936	4.631	4.457	-0.280	1.043*	7.832	7.625
	P3436	3.992	5.359	4.018	5.648	4.892	0.455	0.959*	8.629	8.337
	P3550	3.370	5.188	4.184	4.853	4.565	-0.199	1.003*	9.466	7.085
	RCRMH2	3.415	5.474	4.079	4.139	4.326	-0.185	1.031*	7.739	8.157
	Mean	3.419	5.326	3.998	4.627	**4.341**			7.083	7.861

S^2d_i =Deviation from regression, β_i = Regression coefficient, E_1 = Bheemarayanagudi under heat stress, E_2 = Raichur under heat stress, E_3 =Bheemarayanagudi under early spring and E_4 =Raichur under early spring. Note: Testcross hybrids with no significance are not included

It may be noted that, the combined interactions of cycles and population with environment and location (Env. × Loc. × Pop. × Cyc.) were found significant for all the characters except anthesis to silking interval (Table 3), which indicated the diversity of the genotypes and their responses to different environmental situation at test location and inconsistent performance of genotypes for these characters. Haruna *et al.*, (2017) reported highly significant genotype × location interaction for grain yield of the intermediate maturing top-cross hybrids under drought seeks to justify the need for the testing of the hybrids in multiple locations over years before recommendation.

Analysis of Stability parameters

Once the genotype × environment interactions are found to be significant, the next step is to identify stable genotypes, which interact less with the environments. In the present study, Eberhart and Russell (1966) model was used to identify stable genotypes by utilizing the data on heat stress and early spring condition, because the later crop growth stages of early spring trials conducted at Bheemarayanagudi and Raichur were also got exposed to high temperature regimes. Further, the performances of early spring trials were also comparable with the performance of heat stress trials during summer season. Hence, the four environments *viz.*, Bheemarayanagudi under heat stress as E_1, Raichur under heat stress as E_2, Bheemarayanagudi under early spring as E_3 and Raichur under early spring as E_4 were considered for analysis of stability parameters. The data of optimal (*kharif*)

Table 5: Stability parameters (Eberhart & Russell, 1966) for plant height (cm) of selected testcross hybrids involving DH lines from C_1, C_2 and C_3 of MPS 1 and MPS 2 populations evaluated under heat stress and early spring at Bheemarayanagudi (B'gudi) and Raichur during 2018 and 2019.

S. no.	Pedigree	Performance under				General mean	S^2d_i	β_i	Performance under optimal condition	
		Heat stress		Early spring						
		E_1	E_2	E_3	E_4				B'gudi	Raichur
79	CML451/(MPS-2-C1)-DH8	111.15	123.53	122.40	138.03	126.38	-46.25	0.90	212.68	161.62
28	CML451/(MPS-1-C1)-DH64	115.26	120.98	113.72	134.92	115.70	-46.65	0.97	207.80	166.83
36	CML451/(MPS-1-C1)-DH75	118.52	117.95	117.11	127.08	113.18	-48.58	0.96	205.86	162.17
62	CML451/(MPS-1-C3GS)-DH2	112.28	119.79	109.51	138.85	116.63	-18.15	2.25	200.84	161.43
64	CML451/(MPS-1-C3GS)-DH5	109.50	118.96	109.66	135.00	112.75	25.86	2.24	199.45	154.20
71	CML451/(MPS-1-C3GS)-DH29	110.55	128.67	114.18	139.05	118.75	52.32	2.37	214.76	158.19
72	CML451/(MPS-1-C3GS)-DH30	115.55	110.89	113.48	136.05	115.13	35.58	1.98	207.09	152.40
74	CML451/(MPS-1-C3GS)-DH34	114.21	125.63	127.93	143.71	133.13	-12.23	2.63	236.02	174.83
77	CML451/(MPS-1-C3GS)-DH49	105.66	109.70	110.44	132.08	105.25	-42.56	1.98	197.68	149.15
78	CML451/(MPS-1-C3GS)-DH64	115.65	131.68	117.51	136.46	125.88	20.18	1.70	216.15	166.51
94	CML451/(MPS-2-C1)-DH73	113.45	121.11	111.21	141.69	125.00	52.17	2.33	210.53	172.85
96	CML451/(MPS-2-C2GS)-DH2	112.88	135.76	129.66	138.20	137.68	-14.45	1.91	202.85	169.50
97	CML451/(MPS-2-C2GS)-DH3	111.57	121.77	101.82	141.67	124.23	130.34	2.91	207.03	162.26
98	CML451/(MPS-2-C2GS)-DH9	107.29	124.46	119.83	137.59	126.00	-41.06	2.02	216.44	161.18
101	CML451/(MPS-2-C2GS)-DH20	116.24	129.95	114.27	137.87	129.13	4.04	2.32	224.80	162.99
104	CML451/(MPS-2-C2GS)-DH31	109.61	131.96	122.36	137.26	127.50	-11.50	2.00	233.17	171.31
106	CML451/(MPS-2-C2GS)-DH42	106.43	119.32	119.17	135.15	120.75	-39.74	2.29	190.95	160.05
110	CML451/(MPS-2-C2GS)-DH57	122.00	127.52	120.59	137.44	133.50	2.88	1.64	218.83	171.99
111	CML451/(MPS-2-C2GS)-DH60	121.43	134.69	119.20	139.23	138.75	14.91	1.93	214.30	182.48
114	CML451/(MPS-2-C2GS)-DH75	107.01	119.72	116.45	138.31	122.00	-39.36	2.43	200.36	147.76
116	CML451/(MPS-2-C3GS)-DH10	110.88	127.76	111.35	138.48	121.50	52.44	2.34	200.95	156.49
118	CML451/(MPS-2-C3GS)-DH13	113.25	118.71	115.60	140.34	124.38	-42.80	2.23	206.77	159.10
120	CML451/(MPS-2-C3GS)-DH26	101.16	121.82	108.58	138.76	112.25	37.40	2.84	183.76	167.34
122	CML451/(MPS-2-C3GS)-DH35	108.07	107.69	114.49	137.57	113.00	-12.38	2.29	183.30	151.86
125	CML451/(MPS-2-C3GS)-DH45	106.92	113.34	110.67	138.38	113.38	-27.96	2.57	209.67	163.36
128	CML451/(MPS-2-C3GS)-DH62	105.77	121.82	115.19	139.13	117.75	-26.47	2.63	199.09	168.78
129	CML451/(MPS-2-C3GS)-DH68	112.33	126.83	109.35	135.98	119.63	19.79	2.04	201.42	172.48
Checks	900MG	111.18	129.42	112.45	129.91	120.95	23.02	0.81*	218.92	155.15
	DKC9108	100.05	112.01	121.95	133.40	116.63	87.21	1.36*	218.36	170.46
	NK 6240	108.89	118.55	109.49	134.60	117.63	-41.70	1.31*	206.13	162.80
	P3550	137.85	153.35	149.19	148.72	151.36	-48.73	0.64*	241.42	183.14
	P3436	128.70	136.29	130.25	141.72	136.50	-28.34	0.89	240.89	173.74
	RCRMH2	129.76	134.81	136.11	139.79	136.38	-47.06	0.87	235.79	180.36
	Mean	114.10	123.07	116.33	136.18	**122.42**			209.90	163.49

S^2d_i =Deviation from regression, β_i = Regression coefficient, E_1 = Bheemarayanagudi under heat stress, E_2 = Raichur under heat stress, E_3 =Bheemarayanagudi under early spring and E_4 =Raichur under early spring. Note: Testcross hybrids with no significance are not included

condition were very high and could mislead the stability of genotypes; therefore, it was not included in the stability analysis, but the corresponding genotypes performance under optimal situation was used for comparison only. The results are presented in Table 4 to 6 on the characters which had significant G × E variances and corresponding stability parameters *viz.*, deviation from regression (S^2d_i), regression coefficient (β_i) and mean (X_i).

Stability parameters for grain yield

The Analysis of stability parameters for grain yield (t ha^{-1}) of maize testcrosses involving 111 DH lines derived from C_1, C_2 and C_3 of MPS 1 and MPS 2 populations and six checks (Table 4) shows that, the testcross CML451/(MPS-1-C2GS)-DH7 was identified as stable, as it recorded high mean grain yield (5.476 t ha^{-1}) than population mean (4.341 t ha^{-1}), β_i value nearer to unity (0.905) and non-significant S^2d_i (-0.295) across four environments (E_1, E_2, E_3 and E_4). This DH testcross hybrid produced higher grain yield and possessed

Table 6: Stability parameters (Eberhart & Russell, 1966) for number of kernels per cob of selected testcross hybrids involving DH lines from C_1, C_2 and C_3 of MPS 1 and MPS 2 populations evaluated under heat stress and early spring at Bheemarayanagudi (B'gudi) and Raichur during 2018 and 2019.

S. no.	Pedigree	Performance under				General mean	S^2d_i	β_i	Performance under optimal condition	
		Heat stress		Early spring						
		E_1	E_2	E_3	E_4				B'gudi	Raichur
48	CML451/(MPS-1-C2GS)-DH30	246.89	337.75	329.94	398.15	346.50	-1026.51	1.01	403.54	359.88
94	CML451/(MPS-2-C1)-DH73	269.71	341.43	324.82	369.70	354.70	-1020.10	0.93	381.89	376.66
112	CML451/(MPS-2-C2GS)-DH65	312.70	407.04	344.70	391.41	419.93	-1020.36	0.92	363.02	391.75
12	CML451/(MPS-1-C1)-DH34	197.64	241.50	297.07	367.48	230.68	-960.11	0.95	352.03	381.38
22	CML451/(MPS-1-C1)-DH50	204.65	247.40	298.86	360.75	244.25	-1021.03	0.95	358.61	344.14
53	CML451/(MPS-1-C2GS)-DH42	213.70	295.06	316.71	385.17	302.63	-1029.64	0.94	380.67	350.80
125	CML451/(MPS-2-C3GS)-DH45	192.55	278.44	318.66	382.68	283.78	-1029.21	0.90	350.51	345.66
4	CML451/(MPS-1-C1)-DH11	128.92	246.82	302.29	373.93	223.93	96.24	1.66	325.32	344.72
15	CML451/(MPS-1-C1)-DH38	103.94	220.35	304.43	377.00	207.45	-112.64	1.63	393.35	360.71
19	CML451/(MPS-1-C1 -DH46	90.68	242.32	313.80	380.15	226.13	1349.92	1.92	387.12	369.05
38	CML451/(MPS-1-C2GS)-DH5	137.30	211.60	303.53	376.01	243.30	189.84	1.51	328.23	319.34
44	CML451/(MPS-1-C2GS)-DH21	190.16	279.72	327.93	392.35	317.45	-164.77	1.52	384.67	325.64
66	CML451/(MPS-1-C3GS)-DH8	125.58	286.30	313.88	369.54	273.05	-567.03	1.77	375.78	339.60
69	CML451/(MPS-1-C3GS)-DH19	111.09	327.48	333.34	384.64	306.03	1129.73	1.98	398.67	366.84
71	CML451/(MPS-1-C3GS)-DH29	143.96	283.84	308.50	393.99	293.78	-729.07	1.92	353.12	332.66
72	CML451/(MPS-1-C3GS)-DH30	181.01	259.13	293.27	370.20	271.23	-142.50	1.32	331.63	316.21
77	CML451/(MPS-1-C3GS)-DH49	118.30	317.74	330.23	372.22	294.53	1019.70	1.84	315.07	364.90
97	CML451/(MPS-2-C2GS)-DH3	219.98	302.18	340.08	387.92	347.20	18.56	1.44	337.79	358.02
102	CML451/(MPS-2-C2GS)-DH28	205.77	323.71	323.35	380.75	343.23	-984.48	1.25	369.36	332.50
104	CML451/(MPS-2-C2GS)-DH31	78.89	308.57	319.60	395.81	306.48	-567.95	2.49	328.89	377.39
108	CML451/(MPS-2-C2GS)-DH48	182.10	343.43	318.56	375.84	325.00	-152.14	1.39	386.18	366.27
115	CML451/(MPS-2-C3GS)-DH1	110.64	322.23	318.54	403.41	288.10	818.09	2.45	378.80	396.54
119	CML451/(MPS-2-C3GS)-DH22	160.39	293.89	313.99	374.06	271.08	272.68	1.48	365.18	363.43
129	CML451/(MPS-2-C3GS)-DH68	206.70	315.77	303.22	380.93	291.45	-7.87	1.27	387.68	336.84
Checks	DKC9108	153.12	284.04	324.25	371.93	288.78	-411.65	1.47	398.33	331.32
	900MG	233.14	271.50	305.90	365.73	293.14	-805.48	0.85*	365.17	345.68
	NK 6240	242.29	309.15	308.16	364.55	306.24	-915.18	0.80*	343.46	325.82
	P3436	266.68	339.89	342.31	421.69	352.95	-885.26	1.13*	406.44	389.48
	P3550	210.35	321.53	351.40	412.20	336.38	-957.97	1.48*	443.57	335.87
	RCRMH2	198.72	323.21	296.80	331.60	285.12	321.15	0.74*	365.10	330.36
	Mean	222.02	297.75	316.96	378.53	**303.81**			368.32	358.13

S^2d_i =Deviation from regression, β_i = Regression coefficient, E_1 = Bheemarayanagudi under heat stress, E_2 = Raichur under heat stress, E_3 =Bheemarayanagudi under early spring and E_4 =Raichur under early spring. Note: Testcross hybrids with no significance are not included

acceptable agronomic traits compared to the checks and also performed better under *kharif* season, thus inferred that, the entry would perform better under all the tested environments. The testcross CML451/(MPS-1-C2GS) - DH56 recorded β_i value less than unity (0.637) with non-significant S^2d_i and mean grain yield (4.240 t ha^{-1}) lesser than population mean (4.341 t ha^{-1}); similarly, the check 900MG also recorded β_i value nearer to unity (0.975) with non-significant S^2d_i (-0.284) and mean grain yield (4.014 t ha^{-1}) lesser than population mean (4.341 t ha^{-1}). Hence, these two entries were categorized as adaptable to unfavourable or resource poor environments. Out of 111 testcrosses, nine testcrosses recorded β_i values greater than unity with non-significant S^2d_i and high mean grain yield than population mean, hence were categorized as adaptable to favourable or resource rich environments.

The 89 testcrosses exhibited significant differences for deviation from regression and they were classified as non-significant, it would suggest that the behaviour of these genotypes was unpredictable (Table 4). These results are in accordance with the findings of Archana *et al.*, (2018), who identified the hybrid, ZL11953 × VL1032 as stable for grain yield when evaluated under heat stress and optimal conditions. Divya *et al.*, (2019) reported two hybrids *viz.*, ZH16878 and ZH16930 as stable hybrids for grain yield per hectare which were well adapted for all the locations under high temperature regimes. Similarly, Pavani *et al*., (2019) also identified the hybrids *viz*., RCRMH-12 and RCRMH-4 as stable and superior for grain yield under heat stress and optimal conditions. Patil (2021) also identified two stable hybrids from MPS 2 population, whereas, none of the stable hybrids were identified from MPS 1 under heat stress condition.

Stability parameters for plant height

The plant height (cm) is the important trait indicating heat stress tolerance; because heat stress reduces internodal elongation, which results in reduced plant height and also ear height in the same proportion (Weaich *et al*., 1996, Zaidi *et al*., 2016 and Nisa *et al*., 2019). The analysis of stability parameters for plant height (Table 5) among the 111 DH testcrosses revealed that, one testcross CML451/(MPS-2-C1)-DH8 ($\bar{x}$ = 126.38, β_i = 0.90, S^2d_i = -46.25) along with two checks P3436 ($\bar{x}$ = 136.50, β_i = 0.89, S^2d_i = -28.34) and RCRMH2 ($\bar{x}$ = 136.38, β_i = 0.87, S^2d_i = -47.06) exhibited higher mean values than the population mean, regression coefficients nearer to unity and non-significant deviations from regression when compared to other genotypes. Therefore, these testcrosses were categorized as stable across four environments (E_1, E_2, E_3 and E_4). The testcross, CML451/(MPS-2-C1)-DH8 also performed better under *kharif* season, thus it was concluded that, the entry would perform better under all the tested environments. Whereas, 11 testcrosses recorded higher mean values than the population mean, β_i value greater than unity and non-significant S^2d_i, which indicated that, these were well adapted to favourable or resource rich environment. Two testcrosses recorded less mean values than the population mean (122.42 cm), β_i value nearer to unity with non-significant S^2d_i, which indicated that, these were well adapted to unfavourable or resource poor environment. The 84 testcrosses and four checks (Table 5) were unpredictable since these hybrids possessed significant S^2d_i values. The present investigation are in agreement with the results of Archana *et al.,* (2018), who evaluated 24 hybrids under heat stress across three locations and identified ZL132102 × VL1033 and VL1011 × VL1033 as stable hybrids for plant height. Sowmya *et al*., (2018) found DMH 100-6 and check hybrid Bio 9681 as stable across three locations and two seasons (summer and *kharif*) for plant height. Divya *et al*., (2019) reported two hybrids, ZH16963 and D2244 as stable for plant height across locations under high temperature regimes. Likewise, Pavani *et al*., (2019) also identified RCRMH-5, RCRMH-12 and RCRMH-2 as stable hybrids for plant height under summer and *kharif* seasons.

Stability parameters for 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, Nisa *et al*., 2019 and Waqas *et al*., 2021). The stability parameters for number of kernels per cob of maize testcrosses involving 111 DH lines are presented in Table 6. Among the 111 testcrosses and six checks evaluated, three testcrosses *viz.*, CML451/(MPS-1-C2GS)-DH30 ($\bar{x}$ =346.50, β_i = 1.01, S^2d_i = -1026.51), CML451/(MPS-2-C1)-DH73 ($\bar{x}$ =354.70, β_i = 0.93, S^2d_i = -1020.10), and CML451/(MPS-2-C2GS)-DH65 ($\bar{x}$ = 419.93, β_i = 0.92, S^2d_i = -1020.36) were identified as stable across four environments (E_1, E_2, E_3 and E_4), as they registered high mean number of kernels per cob than population mean (303.81), regression coefficients nearer to unity and non-significant deviations from regression. Further, these testcrosses also produced higher number of kernels per cob during *kharif* as compared to population mean; hence these testcrosses would perform better across all the environments. Whereas, six testcrosses recorded high mean than population mean (303.81), β_i value greater than unity and non-significant S^2d_i, which indicated that, these were adaptable to favourable or resource rich environments. Four testcrosses recorded less mean number of kernels per cob than population mean (303.81), β_i value lesser than unity with non-significant S^2d_i, which indicated that, these were adaptable to unfavourable or resource poor environments. The 84 testcrosses and four checks were unpredictable since these hybrids possessed significant S^2d_i values and they were classified as non-significant (Table 6). These findings are in accordance with the results of Pavani *et al*., (2019), who identified one maize hybrid, RCRMH-12 as stable for number of kernels per cob under heat stress and optimal conditions.

Conclusion

From the present study, the testcross CML451/(MPS-1-C2GS)-DH7 was identified as stable hybrid for grain yield under heat stress and early spring condition and also performed better under *kharif* season. Therefore, this hybrid needs to be tested extensively at different locations under different seasons and situations for its suitability for commercialization besides using the DH line in development of new hybrid combinations and new generation of inbred lines. Use of the best DH testcross hybrids, well targeted to the production environments, could boost maize production among farmers. Similarly, one testcross CML451/(MPS-2-C1)-DH8 found stable for plant height; three testcrosses *viz.*, CML451/(MPS-1-C2GS)-DH30, CML451/(MPS-2-C1)-DH73 and CML451/(MPS-2-C2GS)-DH65 were identified as stable hybrids for number of kernels per cob. Therefore, these hybrids or DH's could be incorporated in breeding stocks for further use in breeding programme.

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Authors' contributions

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

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