Mysore J. Agric. Sci., 57 (2) : 33-39 (2023)

Trait Specific Recombinant Inbred Lines for High Temperature Tolerance in Finger Millet

I. AKHILA ANIL AND Y. A. NANJA REDDY
Department of Crop Physiology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
e-Mail : akhilaanil1525@gmail.com

AUTHORS CONTRIBUTION

I. AKHILA ANIL :
Carried out the experiment, data analysis and drafted the manscript;
Y. A. NANJA REDDY :
Conceptualization, designing and editing

Corresponding Author :
I. AKHILA ANIL
Department of Crop Physiology, College of Agriculture, UAS, GKVK, Bengaluru

Received : August 2022
Accepted : January 2023

ABSTRACT

Agriculture production and productivity are adversely affected due to increasing episodes of high temperature and drought in the changing climate scenario. Finger millet is relatively a drought-tolerant crop but is sensitive to high temperature because it originated in the cool climate of the highlands in Africa. Identification of varieties for high-temperature adaptation could serve against the rising temperature and considering its nutritional importance, the crop can be extended to the non-traditional high-temperature regions. One of the approaches for identifying a genotype tolerant to high temperature could be by exploiting available genetic resources like germplasm lines. However, the use of a stable mapping population is better in identifying better lines over the better parent. Therefore, a study was conducted using 222 recombinant inbred lines (RIL's; F_6) developed for high-temperature tolerance. Differences in temperatures during the crop growth period were achieved by adopting two dates of sowing. The study showed a 1.4 °C rise in temperature during the vegetative to the flowering stage that resulted in a significant decrease in days to flowering, leaf chlorophyll content, the number of productive tillers, mean ear weight, threshing percentage, and grain yield. Correlations showed a significant positive relationship between SCMR, leaf thickness, productive tillers, mean ear weight and threshing percentage with the grain yield under high-temperature condition. Whereas, a significant negative relationship was observed for grain yield with days to flowering and leaf temperature under HT conditions. The principal component analysis (PCA) also showed that grain yield and the number of productive tillers were the most contributing factors to variance. Based on these two traits, significantly HT tolerant RILs over the better parent, PR-202 (PT, 111.2 ± 1.22/m^2 and grain yield, 263.2 ± 3.44 g/m^2) are 6.2.5, 6.5.7, 6.3.9, 6.2.25 and 6.19.15, which can be used in crop improvement or for direct cultivation.

Keywords : Finger millet, High temperature, Recombinant inbred lines, Principal component analysis

GLOBAL mean surface air temperatures are increased by 0.5 °C during the twentieth century and are expected to rise by 1.4 to 3.1 °C by the end of the twenty-first century (Stocker *et al*., 2013). Hence, the high temperature (HT) stress could be one of the major limiting factors for crop production in arid and semi-arid regions. For such regions, millets are better suited; particularly the finger millet (*Eleusine coracana* L. Gaertn.) and is an important crop that is predominantly cultivated in India and Africa. It stands superior amongst cereals and millets, owing to its higher nutritional quality, wider adaptability and subsistence farming. However, high-temperature stress can lead to changes in the plant morphology, physiology and biochemical processes that reduce the plant's growth and development, leading to a loss in grain yield (Sato *et al*., 2002; Vinay Kumar, 2015 and Yogeesh *et al*., 2016). In the case of finger millet, the optimal day and night temperatures for growth and development were reported as 27 to 32 °C and 22 °C, respectively, any increase in temperatures beyond 32 °C affects the flowering and grain filling (Directorate of Millets Development, 2014).

Developing a variety that is tolerant to high temperatures could be of immense help to farmers in high-temperature stress regions. One of the approaches for identifying such lines would be the exploitation of available genetic resources, which is quite challenging and time-consuming (Opole *et al.,* 2018). In this direction, although identification of tolerant lines could be achieved by use of germplasm lines, their availability and time could be of impediment. Therefore, the use of a stable population derived specifically for high-temperature tolerance could be more appropriate. Keeping in mind the future prospects and lacunae in the current scenario, an attempt was made to study the physiology-based field performance within a RIL population of finger millet to identify traits and lines tolerant to HT stress over the better parent.

MATERIAL AND METHODS

A field study was carried out at the University of Agricultural Sciences, GKVK, Bengaluru, Karnataka, India using 222 finger millet RILs (F_6) developed for high-temperature tolerance. The experiment was conducted in an augmented block design with single replication in 6 blocks with three check varieties (GPU-28, PR-202 and KJNS-46) in each block. The parental lines of this population were PR-202 (high temperature tolerant) and KJNS-46 (high temperature sensitive). Two different dates of sowing were undertaken, the first and second sowing during January 2019 (D_1/Normal) and February, 2019 (D_2/high-temperature stress), such that the vegetative to flowering phase of the second sown crop coincided with the months of April and May to have high day temperatures. All the recommended practices for finger millet cultivation were followed to raise a good crop. The leaf temperature, leaf thickness and SCMR were measured at the time of 50 per cent flowering. At the crop maturity, yield attributes like a number of productive tillers (No./m^2), mean ear head weight (g/ear) and grain yield (g/m^2) were measured. Principal component analysis was performed to analyze genetic variability among the RILs and to identify the most important traits contributing to the variability. For the identified traits, the RILs performed superior over the better parent were identified. Data were analyzed in R package, MS excel and Past4 software.

RESULTS AND DISCUSSION

In view of achieving differences in temperature during the crop growth period, sowing dates were managed as D_1 (normal) and D_2 (high temperature, HT). In the present study, the mean maximum and minimum ambient temperatures (from sowing to 50 Per cent flowering) were 32.3 °C and 18.6 °C, respectively, for D_1, and a higher temperature of 33.7 °C and 19.6 °C, respectively, for the D_2 crop. The D_2 sown crop experienced a higher day temperature of 1.4 °C as compared to the D_1 crop (Fig.1), which is expected to reduce the yield attributing traits and grain yield. Plant response for such HT was reported to be highest during flowering and at the early seed filling phase (Djanaguiraman and Prasad, 2014) and genetic variability for HT tolerance exists (Djanaguiraman *et al*., 2017). In fact, previous research reported that a mean HT of 35.6 °C resulted in a 43.0 per cent decrease in grain yield of finger millet when compared to 32.5°C (Anonymous, 2013). However, finger millet will not be affected considerably up to a temperature of 30 °C during the reproductive phase,

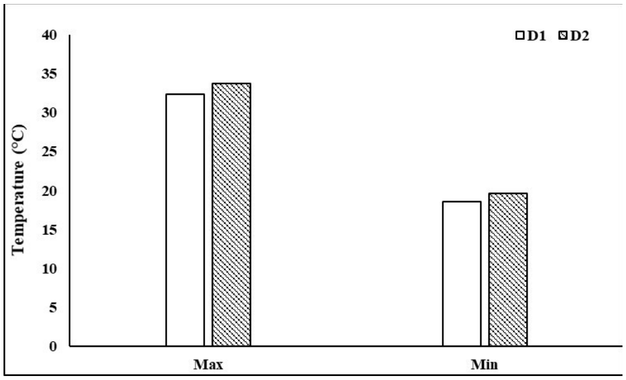

Fig. 1: Mean maximum and minimum temperature from sowing to 50% flowering in January sown (D_1) and February sown (D_2) crop

above which it significantly decreases the grain yield (Ramya and Nanja Reddy, 2018).

In view of this, it would be pertinent to identify HT tolerant genotypes. The existence of genetic variability is the primary criterion for selection (Opole *et al.,* 2018). Such variability existed in the RIL population for physiological and yield attributes (Table 1) (Djanaguiraman *et al*., 2017). The RILs differed significantly for all the traits studied both under normal and HT conditions. The variability (mean sum of squares) in the RIL population was higher under HT conditions (D_2) for days to flowering, leaf temperature, SCMR and productive tillers (Table 1), indicating that individual RILs differ in their response to the HT (Yogeesh *et al*., 2016; Opole, 2018). The higher variability under HT provides an opportunity for the selection of RILs for a given trait, such as early duration or higher productive tillers per hill, to achieve higher grain yield under HT conditions (Yogeesh *et al*., 2016). The variability in grain yield of RILs was less under HT than under normal temperatures (Table 1), implying that the potential expression in terms of grain yield did not differ significantly under HT. However, trait selection is possible, which results in a higher grain yield.

There was a considerable variability noticed among RILs for days to flowering, leaf temperature, leaf thickness and SCMR under high-temperature condition which have ultimately resulted in decreased yield attributes and grain yield as compared to the normal conditions (Fig. 2). Research on other cereals, reported that HT affects the photosystem II quantum yield, photosynthetic rate, pigment system and grain yield (Sunoj *et al*., 2016). The mean leaf temperature and leaf thickness increased under HT conditions as compared to normal conditions (Fig. 2). Under HT conditions, plants might have developed thicker leaves to reduce the loss of water compared to the normal condition.

The extent of the stressful effect of HT on different traits can be studied by the degree of association. Under HT conditions, a significant positive correlation was observed for grain yield with SCMR, leaf

TABLE 1

Mean sum of squares (ANOVA) for phenology, physiological and yield parameters in finger millet sown during January (D_1) and February (D_2), 2019

Source of variation	Df	Day to 50% flowering		Leaf temperature (ºC)		SCMR values		Leaf thickness (mm)		Productive tillers (No./ m²)		Mean ear head weight (g/ear)		Threshing (%)		Grain yield (g/m²)	
		D1	D2	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2
RIL's (Ignoring blocks)	224	2.78 **	36.0 **	1.69 **	2.21 **	5.64 **	6.3 **	0.0006 **	0.0004 **	360.5 **	369.5 **	1.73 **	1.56 **	49.1 **	55.7 **	34506 **	23433 **
Checks	2	26.9 **	255.2 **	5.95 **	11.0 **	51.0 **	6.5 **	0.0024 **	0.004 **	297.1 **	365.5 **	4.31 **	4.82 **	758.7 **	614.6 **	81101 **	63144 **
RILS's Vs checks	1	6.31 **	53.3 **	23.4 **	18.4 **	0.23 ns	2.17 **	0.02 **	0.02 **	10.3 ns	350.5 **	0.95 **	0.31 **	22.6 **	20.2 ns	19206 **	2954 **
RIL's	221	2.4 **	34.0 **	1.56 **	2.05 **	5.25 **	5.79 **	0.0005 **	0.0003 **	362.6 **	369.7 **	1.71 **	1.54 **	246.9 **	50.8 **	34154 **	23166 **
Mean		98.8	98.3	27.8	29.3	29.5	27.6	0.30	0.32	117.0	104.4	2.98	2.59	78.4	74.0	294.5	218.6
SE±		0.59	0.6	0.29	0.48	0.44	0.45	0.01	0.01	2.45	4.1	0.08	0.09	1.89	2.73	9.37	8.34
CV		0.48	0.49	0.84	1.32	1.2	1.31	1.63	1.30	1.68	3.14	2.07	2.93	1.93	2.96	2.57	3.07

TABLE 2

Correlation between phenological, physiological and yield attributes in RIL population of finger millet for heat response in normal (D_1) and HT (D_2) conditions

	DFF	L.Temp	SCMR	L.Thick	PT	MEHW	Th%	GY
DFF (D_1)	1.000							
DFF (D_2)	1.000							
L.Temp (D_1)	0.221	1.000						
L.Temp (D_2)	0.421	1.000						
SCMR (D_1)	-0.223	-0.533	1.000					
SCMR (D_2)	-0.496	-0.596	1.000					
L.Thick (D_1)	-0.135	-0.198	0.362	1.000				
L.Thick (D_2)	-0.210	-0.259	0.359	1.000				
PT (D_1)	-0.104	-0.380	0.559	0.345	1.000			
PT (D_2)	-0.464	-0.453	0.578	0.309	1.000			
MEHW (D_1)	-0.294	-0.632	0.759	0.415	0.656	1.000		
MEHW (D_2)	-0.491	-0.667	0.780	0.423	0.617	1.000		
Th% (D_1)	-0.174	-0.441	0.477	0.253	0.449	0.589	1.000	
Th% (D_2)	-0.350	-0.437	0.517	0.281	0.454	0.637	1.000	
GY (D_1)	0.044	-0.006	0.024	-0.007	0.114	-0.017	0.055	1.000
GY (D_2)	-0.519	-0.639	0.768	0.431	0.751	0.964	0.691	1.000

(DFF: Days to 50% flowering, L.Temp: Leaf temperature (°C), SCMR: SPAD chlorophyll meter reading, L.Thick: leaf thickness, PT: productive tillers (No.m^{-2}), MEHW: Mean ear head weight (g/ear), Th%: Threshing %, GY: Grain yield (g/m^2)).

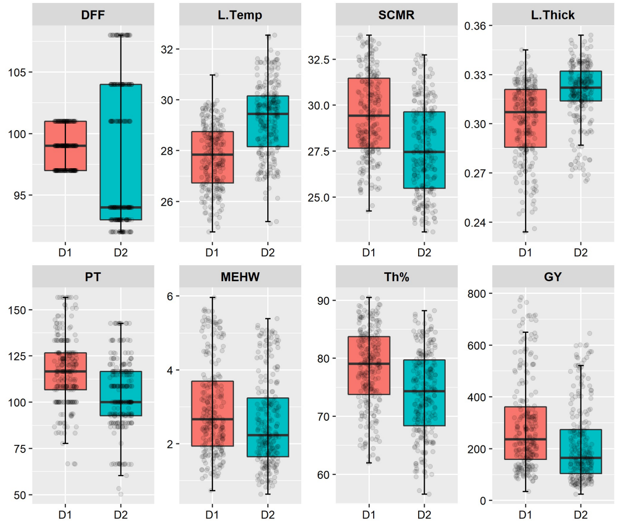

Fig. 2 : Variability in RILs for different traits between January (D_1) and February (D_2) sowings (DFF: Days to 50% flowering, L.Temp: Leaf temperature (°C), SCMR: SPAD chlorophyll meter reading, L.Thick: leaf thickness, PT: productive tillers (No.m^{-2}), MEHW: Mean ear head weight (g/ear), Th%: Threshing %, GY: Grain yield (g/m^2)). The slate colour dots in boxes indicate the distribution of RIL's, and central line in the given box is the mean for that parameter)

thickness, productive tillers, mean ear-head weight and threshing percentage (Table 2). The relationship between the days to flowering and leaf temperature with grain yield was significantly negative under HT conditions (Table.2; Chaudhari and Acharya, 1969). This suggests that flowering is sensitive to HT (Jukanti *et al.*, 2017) and short duration lines are preferable for HT conditions.

Principle Component Analysis

The principal component analysis is one of the best multivariate statistical tools to analyze large populations in order to identify the variability in given traits or genotypes. Principal component-based bi-plots reveals a group of traits, and cluster of genotypes for combining grain yielding potential (Mvuyekure *et al.*, 2018). Scree plot, based on the eigenvalues explains the percentage of variance associated with each principal component (Ladumor *et al.*, 2021). Our results of PCA analysis showed that the contribution for total variance by PC1 (99.09%) was highest followed by PC2 (0.68%; Fig. 3A). Similarly, Ahmad and Mahmood (2015) have reported 99.8 per cent contribution by PC1 and PC2 for total variance. The degree of association of PC1 is strongly associated with grain yield (0.994) and PC2 with productive tiller (0.991). This indicates the number of productive tillers is the most important component of grain yield in finger millet (Chaudhari and Acharya, 1969).

Plotting PC1 against PC2 resulted in the grouping of RILs in 4 classes (quadrant I, II, III and IV) as shown in Fig 3B. RILs in quadrant I recorded positive values of grain yield and negative values of productive tillers.

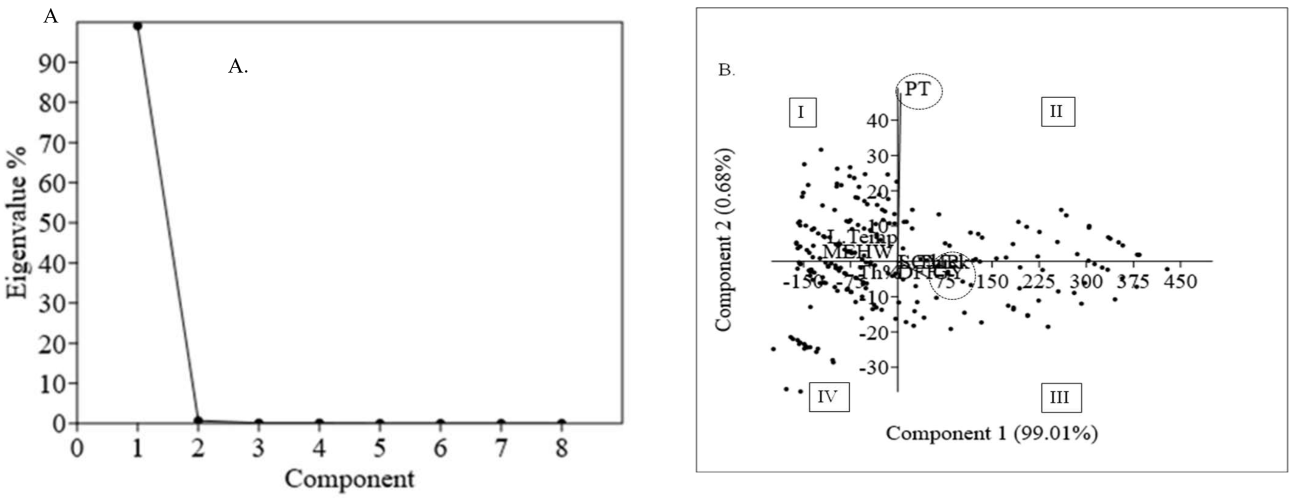

Fig. 3 : Scree plot showing the division of principal components based on eigenvalues (A) and biplot showing the distribution of RILs based on grain yield and productive tillers under high-temperature condition (Component 1 and 2 refers to grain yield and productive tillers respectively, in scree plot)

Similarly, RILs in quadrant II recorded positive values of both grain yield and productive tillers, quadrant III recorded negative values of grain yield and positive values of productive tillers and RILs in quadrant IV recorded negative values of both grain yield and productive tillers. The RILs in quadrants I and IV can be used for grain yield and productive tillers respectively can be utilized in crop improvement by trait specificity. The RILs in quadrant II (Fig 3B) can be considered for direct cultivation. Based on the higher values over the better parent, PR-202 for productive tillers (111.2 ± 1.22/m^2) and grain yield (263.2 ± 3.44 g/m^2), the significantly superior RILs are 6.2.5, 6.5.7, 6.3.9, 6.2.25 and 6.19.25. These identified tolerant RILs can be used for further crop improvement with respect to high-temperature tolerance.

The present investigation on RILs of finger millet revealed a 1.4^{o}C rise in day temperature under HT conditions during the vegetative to flowering stage showed a significant reduction in grain yield and the existence of higher genetic variability among the RILs as compared to the normal condition. The study from principle component analysis showed that the grain yield and productive tiller are the most contributing components to total variance. The RILs performed superior over the better parent, PR-202 for these two traits are 6.2.5, 6.5.7, 6.3.9, 6.2.25 and 6.19.15. These RILs can be used in crop improvement or for direct cultivation under HT conditions.

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