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Research Article

Use of infrared thermography imaging for assessing heat tolerance in high and low iron pearl millet lines

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Abstract

In the arid regions of Asia and Africa, pearl millet serves as a staple source of dietary energy and mineral micronutrients for millions of people. These regions are more vulnerable to increased temperature. The availability of rapid and efficient screening tools based on the relevant non-destructive quantifiable traits would facilitate pearl millet improvement for heat tolerance. The objective of this study was to evaluate pearl millet lines with contrast micronutrients for heat tolerance using infrared thermal imaging, a rapid proxy-canopy (panicle and flag leaf) temperature measurement. Results showed the highly significant genotypic differences between high-Fe and low-Fe genotypes for grain Fe and Zn densities and agronomic traits. Both high-Fe and low-Fe group genotypes differed significantly for panicle temperature depression (PTD) during high- vapor deficit (VPD) at stigma stage (3.0 to 6.73°C). PTD values were positive across all genotypes during stigma stage and were very low or negative during the low-VPD. Cooler canopy temperature (high-PTD) was observed during stigma stage rather than seed-set stage at higher-VPD in both high-Fe and low-Fe genotypes. The cooler temperature achieved by panicle might be helpful in maintaining stigma receptivity for longer periods in the female parents, whereas in male parents it might be helpful in maintaining pollen viability for longer periods. Flag leaf temperature (FTD) was cooler than PTD at both high-VPD and low-VPD as well in both stigma (less by 2.1°C) and grain-filling stage (less by 2.7°C), again signifying that the reproductive parts are more prone to heat stress as compared to vegetative parts. Since, thermal imaging discriminates the heat stress and non-stress canopies, this can serve as a proxy canopy temperature tool for heat stress tolerance screening in pearl millet.

Key words

Infrared image, heat tolerance, iron, zinc, pearl millet

INTRODUCTION

At present, crop breeding program challenges the development of new series of cultivars that maintain higher yields and maintains good minerals content under the climate change scenario. Pearl millet (*Pennisetum glaucum* (L.) R. Br.) grown mostly on marginal areas of the arid and semi-arid tropical regions of Asia and Africa. India is the largest producer of this crop with >8 million ha area and 8.5 million tons of grain production (Yadav *et al.*, 2012). Generally, pearl millet cultivated in the rainy season (June-October) in India. Its cultivation is expanding as an irrigated summer season (February-June) crop in parts of Gujarat (Mula *et al.*, 2009), as well as in Rajasthan and Uttar Pradesh where assured irrigation is feasible (Reddy *et al.*, 2013). Summer season characterized by a very high temperature conditions while in the rainy season have moderate temperature. As a consequence, the reproductive phase of this crop gets

exposed to higher VPD conditions during the April-May (up to 45°C in summer). Also, this crop had showed to >60% set seeds in field conditions while day temperature had gone up to 42°C, whereas the ability of seed set decreased drastically after 42°C (Gupta *et al.*, 2015). This coincidence of flowering with high temperature in summer resulting in reduced seed set thereby expected lower grain yield, mostly in early-maturing hybrids. Thus, medium to long duration hybrids are recommended for high-inputs summer crop. Due to this limited hybrid options for summer, different types of pearl millet parents including mineral dense genotypes were evaluated to identify sources of high temperature tolerance. Farmers are failed to establish adequate plant populations is often associated with high temperatures during the germination and seedling establishment period including the rainy season in Rajasthan. Therefore, high temperatures have

relevance at both seedling and reproductive stages of pearl millet (Yadav and Rai, 2013). In several parts of Asia and Africa, pearl millet serves as a major source of dietary energy and mineral micronutrients. Breeding lines and hybrid parents with high and low levels of Fe density have been identified at ICRISAT in partnership with HarvestPlus biofortification program. Identifying high Fe density with high yielding capacity under high VPDs would offer dual benefits (high-iron and heat tolerant) in summer seasons for farmers in semi-arid and arid environments.

Canopy temperature depression (CTD) have been used as heat and drought tolerance indices, and is a measure of difference between air temperature and canopy temperature, to understand plant water status, practical implications on irrigation scheduling etc (Balota *et al.*, 2007). Genotypes with higher CTD were considered tolerant for heat stress in self-pollinated crops like wheat (Amani *et al.*, 1996; Reynolds *et al.*, 2001). Even short time effect of heat stress during reproductive stage would affect on stigma, pollen formation and its viability, and will directly lead to poor seed set (Matsui *et al.*, 2007). In rice, the panicles under high VPD condition found to be lesser panicle temperature by 0.4-1.5°C in china (Yoshimoto *et al.*, 2005), 2.8-5.2°C in north western Mexico (Ayeneh *et al.*, 2002) and 4-6.8°C in Riverina region of North South Wales, Australia compared to ambient temperature as a result of transpiration cooling (Matsui *et al.*, 2007), a mechanism to avoid heat stress. In pearl millet no such information is available so far. Hence the present study was aimed at the genotypic differential response to panicle temperature depression at two growth stage, at stigma-stage and 10 days after stigma-stage (seed formation) of selected lines that contrasted for Fe density. If a genotype with high-Fe density, high panicle temperature depression under high VPD condition identified, and then the same genotype can be used in both heat tolerance and Fe biofortification breeding programs. This is a preliminary study, in which we intend to understand whether high-Fe and low-Fe inbred parental lines were different for transpiration cooling which was measured through a proxy-canopy temperature measurements from IR-images.

MATERIAL AND METHODS

This study consisted five high-Fe and two low-Fe inbred lines (Table 1) for screening panicle/flag-leaf thermal images at two important growth stage namely, flowering (stigma stage) and seed-filling (10 d after stigma stage).

All these lines were planted in two row plots of 4 m long with 60 cm spacing between the rows and 10-15 cm spacing between plants in Alfisols field condition during 2015 summer season at Patancheru (17°30'N; 78°16'E; Altitude 549 m). Standard agronomic practices were followed for good crop growth, and plots were irrigated 8-10 days interval to ensure no moisture stress. The open pollinated main panicles of five random plants with good seed set from each plot were harvested at or after physiological maturity, all these harvested panicles were well sun dried on tarpoulaine sheet for 12-15 days, stored in cloth bags, and hand threshed and the grains cleaned of any glumes as well as foreign matters, if any, to produce grain samples for laboratory analysis.

The data logger (Lasca Electronics Inc. UK) were mounted at par with plant height and closer to each plot for regular record of the air temperature and relative humidity at 5 min intervals throughout the measurement period. Data loggers were dangled in thermocol box (to protect it from rain and direct sunlight) with holes over all the sides for free air flow (Fig.1). In each plot, three to five randomly selected plants were tagged for capturing images at stigma stage (ST) and grain filling stage (GF) (Fig. 2). Panicle images were captured in all the genotypes and flag-leaf images were also capture in ICMA 98222 and ICMR 1201. Each plant panicle/flag-leaf images were captured in three different directions (in each direction three images) during morning hours (low-VPD) between 7.30-8.30 am and afternoon hours between 2.0-3.0 pm (high-VPD) (Fig. 3). Infrared images were captured using *Fluke Thermography (Ti55FT model)* camera. Images were analyzed using *SmartView* software through spotting panicle area using an image spotting option. Panicle and flag-leaf temperature depression were estimated by deducting panicle/flag-leaf temperature from the air temperature.

Panicle Temperature Depression (PTD) = (Air temperature – Panicle temperature)
Flag leaf Temperature Depression (FTD) = (Air temperature – Flag leaf temperature)

Grain yield per plant, plant height, panicle length, panicle girth, spikelet stalk length (stalk length between base of seed and its attachment position on fascicle of panicle), 1000-grain weight were also recoded from all the plants used for IR image measurements.

Table1. Pedigree details of genotypes used in the experiment.

Genotype	Pedigree
ICMA 98222	ICMB 98222 backcrossed to 842A cytoplasm source (A1)
ICMB 98222	ARD-288-1-10-1-2 (RM)-5
ICMA 02333	ICMB 02333 backcrossed to 81A cytoplasm source (A4)
ICMB 02333	(BSECBPT/91-39 x SPF3/S91-116)-15-2-1-2
ICMR 1201	(MC 94 C2-S1-3-2-2-2-1-3-B-B x AIMP 92901 S1-488-2-1-1-4-B-B)-B-2-2-2
ICMB 92888	(843B x ICMP5 900-9-3-2-2)-41-2-6-2-2
ICMB 94222	{(843B x (843B x 700651))-11-1-2-B x 1163B} x (ICMB 89111 x ICMB 88004)-3-3



Fig. 1. Data loggers mounted (at plant height) near to the each genotype plots for recording air temperature and relative humidity at specified intervals.

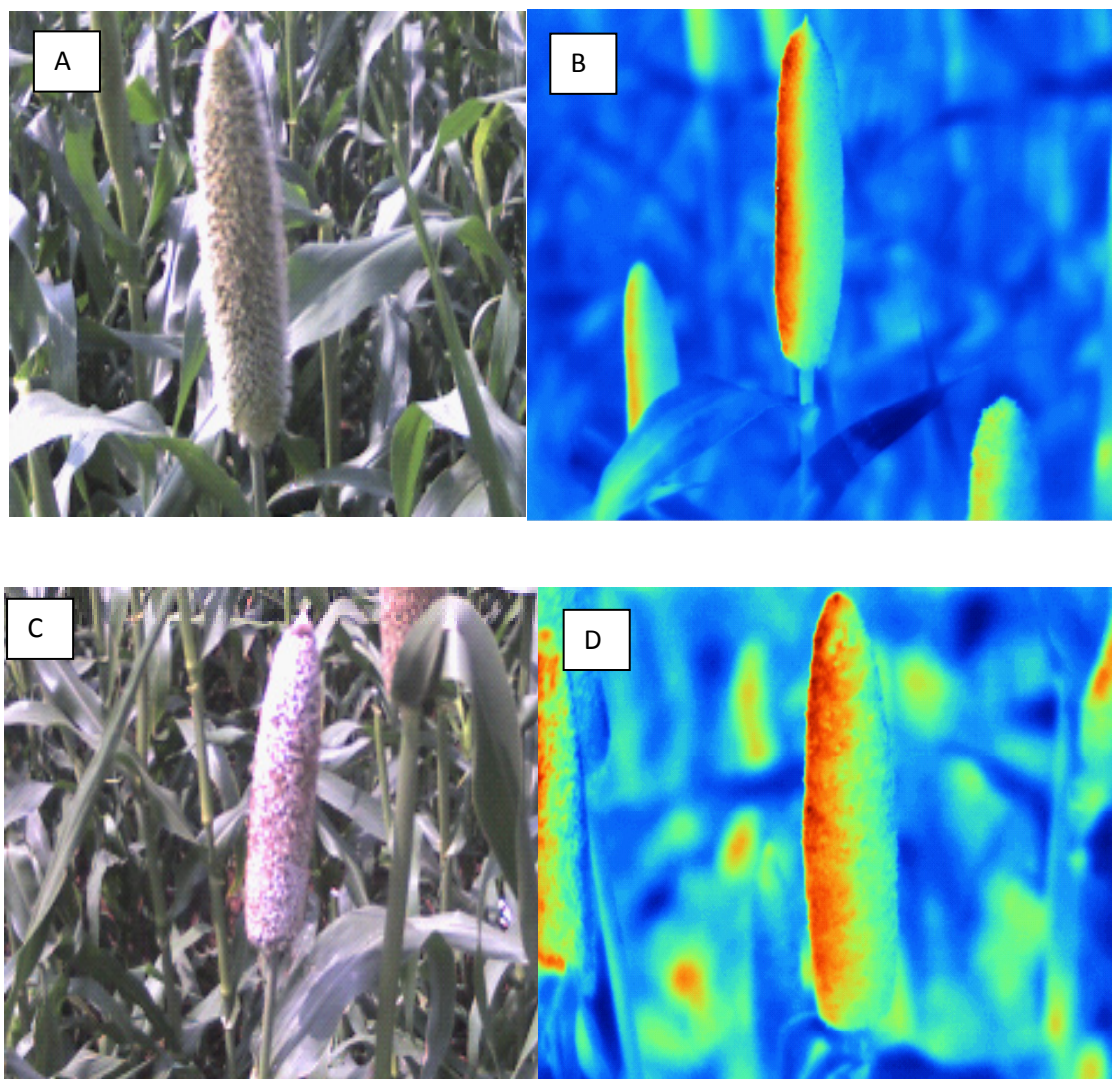


Fig. 2. Visual and thermographic images of pearl millet panicles at stigma stage (A, B) and at seed-set stage (C, D)

Grain iron and zinc densities were estimated using XRF (Govindaraj *et al.*, 2016) at pearl millet breeding lab, ICRISAT, Patancheru. XRF method is scanning based method, in which aluminium sample cups of 30 mm diameter, 36 mm depth and >20 g of grain weight capacity, with polypropylene inner cups sealed at one end with 4 μm Poly-4 XRF sample film. Cups in a batch of 10 were filled with 8-12 g of grain as suggested by Paltridge *et al.* (2012). The cups were shaken to distribute grains evenly

within the cups, which were loaded in the XRF instrument holder, and after analysis the Fe and Zn density display on the monitor attached to XRF machine.

Data analyses were carried out using individual plant observations. Analysis of variance (ANOVA) was performed using PROC GLM program in SAS university edition and principal component analysis (PCA) was performed in R-software.

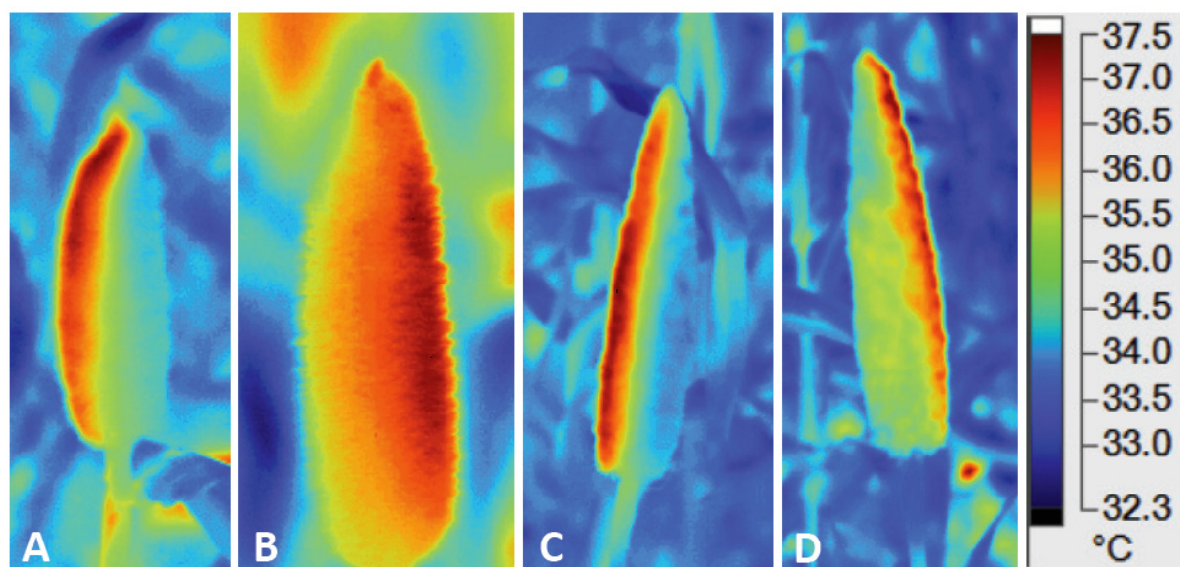


Fig. 3. Infrared (IR) thermographic images of panicles from high-iron line ICMA 98222 at stigma stage (A) low-VPD; (B) high-VPD) and grain-filling stage (C) low-VPD; (D) high-VPD).

RESULTS AND DISCUSSION

Several methods suggested screening breeding materials for heat tolerance, while, thermal infrared imaging is a proven technology for phenotyping the plant canopy traits such as heat stress by measuring the plant canopy temperature (Benavente *et al.*, 2013; Sivasakthi *et al.*, 2017). Present study report for the first time of this tool for pearl millet flowering heat tolerance screening. There were highly significant differences observed between high-Fe and low-Fe genotypes for grain iron (Fe) and zinc (Zn) densities (Table 2). High-Fe genotypes had 79 to 91 mg kg^{-1} Fe and 28 to 57 mg kg^{-1} Zn, and in low-Fe genotypes had 30 to 31 mg kg^{-1} Fe and 16-18 mg kg^{-1} Zn. Similarly, a significant variability observed for agronomic traits, days to 50% flowering, 1000-grain weight, plant height, panicle length, panicle girth, spikelet length, except grain yield plant⁻¹ (Table 2).

At stigma stage, significant variability observed for PTD during both low-VPD and high-VPD (Table 3). The PTD values were positive and high across all genotypes at high-VPD, which varied from 3.00 to 6.73°C. While PTD were low or negative during the low-VPD, it varied from -2.03 to 0.31°C. This might be due to that all the selected

genotypes had higher transpiration rate under high-VPD conditions which leads to transpiration cooling, a possible physiological mechanism to avoid high tissue temperature (Benavente *et al.*, 2013; Sivasakthi *et al.*, 2017). This variation is encouraging as within just seven genotypes, if large sets of inbred and hybrids are evaluated then there will be a great chance to identify potential material for further breeding. At seed-set stage, there were significant differences observed for PTD during low-VPD as well as high-VPD (Table 3). However, PTD values were lower than as observed at stigma stage, which varied from -1.03 to 3.97 at high-VPD and from -1.55 to 2.43 during low-VPD. Among the studied high-Fe genotypes, ICMA 02333 and ICMB 02333 maintained positive PTD during both low- and high-VPD conditions at seed-set stage. These results indicate ICMA 02333 and ICMB 02333 may be able to undergo transpiration cooling since from morning hours. Particularly at seed-set stage ICMB 02333 may have an efficient cooling which is worth testing further.

The difference between average of low and high-Fe groups was significant at stigma stage as well as seed-set stage during high-VPD, but not during low-VPD. This implies that, high-VPD condition/environment could

Table 2. Genotypic variations for micronutrients and yield traits in pearl millet.

Genotype	Fe class	Fe (mg Kg ⁻¹)	Zn (mg Kg ⁻¹)	Days 50% flowering (d)	1000 grain weight (g)	Spikelet length (mm)	Plant height (cm)	Panicle length (cm)	Panicle girth (mm)	Yield per plant (g)
ICMA 98222	High	91	28	42	13	10	115	18	36	47
ICMB 98222	High	79	33	43	11	9	113	16	36	42
ICMA 02333	High	88	34	44	9	9	114	19	32	46
ICMB 02333	High	84	42	46	10	9	118	19	36	40
ICMR 1201	High	86	57	45	8	8	159	26	32	51
ICMB 92888	Low	31	16	45	10	7	88	22	28	41
ICMB 94222	Low	30	18	45	9	7	77	15	26	53
Mean		70	32	44	10	8	112	19	32	46
SE±		3.7	2.8	0.3	10.8	0.5	4.1	1.0	1.3	10.8
F-Probability		<.0001	<.0001	<.0001	0.0024	0.0017	<.0001	0.0002	0.0004	NS

be more appropriate to differentiate low and high-Fe genotypes for PTD. Also, the larger difference between high and low-Fe group was observed at stigma stage than seed-set stage, this further implies that at stigma stage during high-VPD would be considered as critical-point to differentiate low and high-Fe group with respect to PTD. Between high-Fe isogenic pairs, B-lines (male fertile) showed relatively higher PTD than A-lines (male sterile) at stigma stage as well as seed-set stage during high-VPD. The high temperature in summer with no moisture stress is still possible that opening of stomata allow the

plants to reduce canopy or leaf temperature through increased transpiration, indicate genotypic variations for transpiration cooling contributes heat tolerance (Benavente *et al.*, 2013). This pattern of cooler temperature achieved by panicle will help in maintaining the stigma receptivity for longer period by avoiding the stigma drying in the male sterile lines, whereas in male fertile lines it might be helpful in maintaining the stigma receptivity as well as pollen viability for longer period. However, these aspects needed to be validated before large-scale application.

Table 3. Variability for panicle temperature depression (PTD) among the pearl millet lines.

Genotype	Fe-class	Stigma stage						Seed set stage					
		Morning (low-VPD)			Afternoon (high-VPD)			low-VPD			high-VPD		
		Air temp (A)	Panicle temp (P)	A-P (PTD)	A	P	PTD	A	P	PTD	A	P	PTD
ICMA 98222	High-Fe	32.0	31.7	0.31	40.0	35.7	4.26	28.0	28.1	-0.09	37.0	38.0	-1.03
ICMB 98222	High-Fe	32.0	32.0	0.03	40.0	33.3	6.73	28.0	28.4	-0.41	37.0	36.9	0.09
ICMA 02333	High-Fe	28.0	29.6	-1.56	40.0	35.6	4.43	29.0	26.6	2.43	37.0	34.6	2.37
ICMB 02333	High-Fe	28.0	28.6	-0.62	40.0	35.4	4.59	29.0	27.1	1.91	37.0	33.0	3.97
ICMR 1201	High-Fe	26.0	26.2	-0.23	40.0	36.0	3.95	27.5	28.6	-1.07	37.0	36.7	0.28
ICMB 92888	Low-Fe	27.0	29.0	-2.03	39.0	36.0	3.00	28.0	29.1	-1.05	36.0	34.1	1.89
ICMB 94222	Low-Fe	27.0	27.6	-0.60	39.3	35.2	3.82	28.0	29.6	-1.55	36.0	33.3	2.70
SE±		0.18	0.38	0.39	0.25	0.38	0.32	0.09	0.55	0.52	0.10	0.65	0.40
F-probability				<.0001			<.0001			<.0001			<.0001

The high cooler temperature was maintained in the flag-leaf (vegetative part) than that of panicle (reproductive part) temperature in both genotypes (A-line and R-line) (Table 4) as well as both stages of crop (stigma and grain-filling) which implied that the reproductive parts are more prone to heat stress compared to vegetative parts. Similar finding is reported in pearl millet (Gupta *et al.*, 2015). It is also reported that the vegetative stage tolerant to heat stress while reproductive stage (particularly flowering) extremely sensitive to heat stress in rice (Jagadish *et al.*, 2010). Earlier studies explained two ways, (i)

tolerating the stress with the presence of specific genes, (ii) avoidance of heat stress through transpiration cooling provided no water stress persists and escaping with early morning flowering and early maturing (Weerakoon *et al.*, 2008; Ishimaru *et al.*, 2010). It is important to note that, panicles do not have functional stomata as present in leaves, but transpires through epidermal pores which are permanently open and subjected to changes in evaporative demand. Thus, depending on the climatic conditions, the panicle temperature may be higher or lower than the air temperature (Lafarge *et al.*, 2016). The high and low Fe

density groups apart from Fe and Zn density, were also found to be significantly different for spikelet stalk length and panicle girth (**Table 2**). Study in wheat (Calderini and Ortiz-Monasterio, 2003) reported that the grain micro and macro nutrient concentrations in genotypes decreased at grain positions more distal from the rachis. Though there

is a possibility of spikelet stalk length to have a role in Fe, Zn accumulation, it needs to be confirmed with larger number of genotypes in future trials. If similar conclusions are derived, then the reasons behind the correlation may need to be deciphered by observing anatomical structure, thickness etc.

Table 4. Variability for panicle flag leaf temperature depression (FTD) among the pearl millet lines.

Stage	Time	Genotype	Air temp	Flag leaf temp	FTD
Stigma stage	low-VPD	ICMA 98222	25.7	25.9	-0.21
		ICMR 1201	26.0	25.4	0.57
	high-VPD	ICMA 98222	40.1	34.1	6.02
		ICMR 1201	40.0	33.0	6.97
Seed set stage	low-VPD	ICMA 98222	26.6	28.3	-1.72
		ICMR 1201	27.5	27.5	0.06
	high-VPD	ICMA 98222	36.2	33.9	2.26
		ICMR 1201	37.0	33.0	4.02

The present study explores the possibility of developing a new and rapid methodology for heat tolerance screening and helps in identifying new traits like high-PTD to cope with increased temperature. The low canopy temperature can be a good indicator of heat tolerant and may be used as a selection criterion in identifying drought tolerant genotypes. Stigma stage at higher-VPD could be considered as a critical-stage to differentiate among genotypes for heat-tolerance. These results provide a foundation for future research directed at identifying powerful heat tolerant traits using rapid techniques thereby can be helpful to enhance breeding programs for heat tolerance. However, this merits needs further detailed study with a diverse and larger sample in different target environments to understand mechanisms for wider applications in breeding programs.

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