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Characterization of sorghum lines for low-temperature tolerance and reproductive performance in the post-rainy season

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Low night temperatures during the flowering stage in November–December significantly affect sorghum production in the semi-arid regions of India, particularly during critical growth phases. Temperature plays a critical role in sorghum flowering and grain yield, as low temperatures can delay flowering, reduce pollen viability, and result in poor seed set, ultimately lowering yield. To investigate this, a set of experiments was conducted at ICRISAT, India, to characterise 16 sorghum genotypes—including nine varieties and seven hybrid parental lines—under both controlled environment facilities and precision experimental field conditions, with three replications per genotype. The results indicated that the B line 296B and the R line ICSR 149 were the most susceptible to cold stress, while B lines 4111B, 104B, ICSB52, and 185B exhibited greater tolerance. Among the low temperature treatments, Treatment 2 (25 °C/5 °C) had the most adverse impact, recording the lowest values for pollen viability, PHI, and grain yield. Significant positive correlations were observed between grain yield and PHI ($r = 0.74$, $p \leq 0.01$), underscoring PHI's potential as a reliable phenotypic marker for cold tolerance. In field experiments, both genotype and date of sowing (DOS) significantly influenced all measured traits except plant height. Notably, the second sowing date (8th November) resulted in substantial reductions in mean PHI, panicle weight, and grain yield, further highlighting the adverse effects of low temperatures on sorghum performance. To evaluate cold tolerance, indices such as Stress Susceptibility Index (SSI), Stress Tolerance Index (STI), and Yield Stability Index (YSI) were applied. The collective integration of cold tolerance indices concludes Dagdi Solapur and Phule Anuradha are consistent performers across the stress tolerance Indices and should be candidates for the cold-tolerant breeding program. The B line 4111B could be a better replacement of the popular 296B line for cold-tolerant breeding.

Keywords Sorghum, Cold stress, Pollen viability, Panicle harvest index, Controlled environment

Sorghum (*Sorghum bicolor L.* Moench) is a multipurpose crop grown in more than 100 countries globally and ranks fifth among the cereals in the area under cultivation. It is a dietary major staple for 600 million people, especially in Asia and sub-Saharan Africa. In tropical countries like India, grain sorghum is grown in rainy and postrainy seasons. Postrainy sorghum is prized for its food value as it produces clean grain, free from diseases. The postrainy season sorghum covers 45% of cultivated land in India and contributes 32% to sorghum production[1,2]. Sorghum is known for its resilience to drought and heat and is well adapted to semi-arid regions. However, it is more sensitive to low temperatures, particularly during the reproductive stage—a vulnerability that can result in significant yield losses compared to other cereals[3,4]. Optimal temperature for sorghum germination is 21–35 °C and 26–34 °C for vegetative growth and 25˚- 28 °C for reproductive[4,5]. In semi-arid regions of India, Low night temperatures during the post-rainy season negatively affect germination and result in poor seedling emergence and growth and reduce plant vigor[6] , significantly delay flowering and maturity, and ultimately reduce yield[7,8]. Cold stress during flowering induces physiological disturbances such as spikelet sterility, flower abortion, and reduced pollen viability, all of which contribute to poor seed set and ultimately yield loss[9,10] . The deployment

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of cold-tolerant cultivars can help extend the cultivation window and enhance productivity under suboptimal thermal conditions[11].

However, screening for cold tolerance is complex, as genotypic responses vary across growth stages. Many existing protocols on screening methods focus primarily on seedling responses to cold, which may not reflect tolerance during later stages[12]. Furthermore, genotypes that show tolerance to cold at the vegetative stage often fail to exhibit the same tolerance during the reproductive stage[13,14]. This highlights a critical research gap: there is a lack of comprehensive, multi-stage screening approaches under both controlled and field conditions to identify cold-tolerant genotypes, especially for reproductive-stage resilience. Moreover, current breeding efforts are limited by the absence of well-defined phenotypic markers that reliably predict cold tolerance in diverse environments. Therefore, developing a field screening technique that can assess cold tolerance during the reproductive stage and identify potential sources of tolerance is crucial. This approach is vital for improving yield potential and stability, particularly during the post-rainy season. Field screening of genotypes may be affected by confounding environmental factors such as abiotic stresses, which are found to affect the crop in combination, like the cold and drought stresses in the post-rainy season sorghum.

The present study addresses this gap by (i) evaluating 16 sorghum genotypes under three defined cold temperature regimes in a controlled environment; (ii) validating these responses under post-rainy a field experiment in precision research fields at ICRISAT, India; and (iii) identifying key phenotypic traits associated with cold tolerance during the reproductive phase. This dual-environment, multi-trait screening approach aims to enhance selection efficiency and enable targeted breeding of cold-resilient sorghum cultivars suited for marginal and post-rainy season environments. By bridging knowledge from controlled environment physiology and field-based performance, the study contributes novel insights into sorghum adaptation strategies under low temperature stress.

Result

Analysis of variance and mean performance of genotypes in control environment experiment (T1 to T3) and in field experiment (T4 & T5)

Low temperatures affect panicle initiation and development in sorghum. Night temperatures of 13 °C or less during the flowering stage can induce male sterility, reduce pollen viability, and ultimately impact grain formation. Unlike the effects of high temperatures, there is limited information available on the impact of cold temperatures on the reproductive growth of sorghum. In the present study, 16 sorghum genotypes were screened under controlled conditions to assess cold tolerance for traits such as Panicle Harvest Index (PHI), Pollen Viability (PV), grain yield, days to 50% flowering, plant height, and days to maturity were evaluated and in a field experiment for traits Panicle Harvest Index (PHI), grain yield, days to 50% flowering, plant height, seed weight and panicle weight . For control environment experiment Analysis of variance (ANOVA) revealed significant differences for all traits studied ($P \geq 0.01$), except for pollen viability in treatment T1, which was non-significant (Table 1). In case of field experiment, the Bartlett's test was performed to assess the homogeneity of error variances before pooling the data from the two sowing dates. The p-values from Bartlett's test indicated that there were no significant differences in variances between the two sowings for all traits except Panicle Harvest Index. Therefore, a pooled analysis of variance (ANOVA) was conducted to evaluate the effects of genotypes,

Traits	Treatment	Min	Max	Mean	σ^2g	SE	h^2
DTF	T1	59	87	77	200.2**	3.4	94
	T2	62	101	85	470.5**	5.6	93
	T3	49	89	73	229.7**	2.3	98
DTM	T1	88	134	118	357.2**	2.1	99
	T2	91	131	122	323.3**	3	97
	T3	77	117	109	294.9**	4.5	93
PH	T1	61	143	107	1881.5**	6.6	98
	T2	59	122	96	1252.1**	8.1	95
	T3	71	116	99	747.8**	8.4	91
PHI	T1	6	81	51.7	1784.5**	4.76	92
	T2	3	60	34.18	1518.4**	3.9	99
	T3	40	69	59.2	366.7**	7.7	86
PV	T1	85	91	89.1	82.4^{NS}	6.5	48
	T2	45	88	75.6	679**	9.6	84
	T3	90	94	92.2	83.2**	4.3	72
GY	T1	0.8	28.6	13.1	195.2**	1.52	78
	T2	0.4	17.7	7.7	132.71**	1.3	96
	T3	10.8	50.9	33.7	320.9**	5.1	92

Table 1. ANOVA, Mean, Range, Heritability for studied traits in individual cold treatments (T1 to T3). T1 = treatment 1, T2 = Treatment 2, T3 = control treatment, σ^2g = genotypic variance, SE = standard error, $h^{2=}$ heritability broad sense.

Source of Var	df	DTF	PHT	Seed wt	Panicle wt	PHI	GY
Genotype	15	184.68**	15020.4**	1.10**	2.19**	206.28**	1.73**
DOS	1	2410.01**	61.8 NS	4.95**	85.48**	627.26**	56.19**
Geno x DOS	15	309.27 NS	184.3 NS	0.07 NS	0.33 NS	37.38 NS	0.19 NS
CV		3.5	10.4	6.2	23.1	10	25

Table 2. Analysis of variance for genotypes, date of sowing and interaction using field data.

	Panicle Harvest Index % (PHI)				Pollen Viability % (PV)				Grain Yield (g plant^{-1}) (GY)			
Genotype	**T1**	**T2**	**T3**	**Mean**	**T1**	**T2**	**T3**	**Mean**	**T1**	**T2**	**T3**	**Mean**
104 B	56	51	61	**56**	88	84	92	**88**	12.9	17.7	39.8	**23**
185 B	26	9	59	**31**	88	65	90	**81**	4.8	1.3	26.6	**11**
296 B	*	*	40	*	*	*	90	*	*	*	10.8	*
4111 B	80	43	54	**59**	89	65	90	**81**	28.6	10.2	30	**23**
ICSB 52	6	3	57	**22**	88	40	94	**74**	0.8	0.4	36.5	**13**
BARSHI ZOOT	51	27	62	**47**	91	88	94	**91**	13.1	4.7	39.2	**19**
D. SOLAPUR	67	54	60	**60**	91	85	93	**90**	19.5	17.4	30.8	**23**
ICSR 149	*	*	57	**57**	*	*	91	**91**	*	*	33.3	**33**
ICSR 93034	49	14	66	**43**	89	82	94	**88**	12.4	1.8	37.9	**17**
M 35–1	57	60	69	**62**	90	82	94	**89**	14.5	12.7	50.9	**26**
P. JYOTHI	64	60	58	**61**	91	86	92	**90**	8.8	11.3	23.6	**15**
P. VASUDHA	58	36	64	**53**	90	72	92	**85**	11.7	7.3	44.3	**21**
P.ANURADHA	81	59	66	**69**	90	78	93	**87**	19.5	9.5	37.7	**22**
SHANGHAI RED	19	4	53	**25**	90	84	90	**88**	3.1	2	19.4	**8**
SPV 1359	56	45	64	**55**	89	75	93	**86**	18.3	4.8	42.7	**22**
SPV 1411	56	15	59	**43**	85	45	94	**75**	15.5	7.7	36	**20**
	Days to 50% Flowering (days)				**Plant height (cm)**				**Days to Maturity (days)**			
104 B	84	97	71	**84**	87	92	105	**95**	112	128	114	**118**
185 B	72	76	70	**73**	130	97	83	**103**	123	127	110	**120**
296 B	*	*	72	*	60	57	73	**63**	*	*	117	*
4111 B	72	73	68	**71**	115	97	100	**104**	119	118	107	**115**
ICSB 52	76	88	74	**79**	117	95	88	**100**	121	124	111	**119**
BARSHI ZOOT	85	94	85	**88**	112	110	105	**109**	114	130	115	**120**
D. SOLAPUR	69	102	73	**81**	60	65	92	**72**	113	122	110	**115**
ICSR 149	*	*	75	*	100	59	68	**76**	114	126	104	**115**
ICSR 93034	72	69	74	**72**	105	95	97	**99**	117	115	108	**113**
M 35–1	80	83	73	**79**	110	103	110	**108**	123	124	113	**120**
P. JYOTHI	81	96	89	**89**	108	117	117	**114**	122	131	116	**123**
P. VASUDHA	88	94	80	**87**	143	123	118	**128**	134	130	117	**127**
P.ANURADHA	82	79	71	**77**	130	112	117	**120**	117	113	104	**111**
SHANGHAI RED	58	60	48	**55**	82	90	83	**85**	88	90	75	**84**
SPV 1359	82	82	76	**80**	142	121	115	**126**	121	125	110	**119**
SPV 1411	81	84	68	**78**	117	98	103	**106**	116	126	109	**117**

Table 3. Mean performance 16 sorghum genotypes for studied traits in three temperature treatments (T1 to T3).

date of sowing (DOS), and their interaction (Genotype × DOS) for all traits. Genotypes and DOS were significant for all the studied traits except DOS for plant height was non-significant, whereas Genotype × DOS interaction was non-significant for all the studied traits. (Table 2).

The mean performance of the traits studied in both control environment experiments and in filed experiments were mentioned in in Tables 3 & 4 respectively.

Days to 50% flowering

In case of controlled environment experiment Cold stress significantly delayed flowering compared to the control. Across treatments, flowering ranged from 58–88 days in T1, 60–103 days in T2, and 48–89 days in T3,

	Days to 50% Flowering (days)		Plant height (cm)		Seed weight (gm)		Panicle weight (kg)		Panicle Harvest Index % (PHI)		Grain Yield (kg)	
Genotype	T4	T5	T4	T5	T4	T5	T4	T5	T4	T5	T4	T5
104 B	80	90	183	188	3.4	3.2	3.6	2.0	75.6	73.2	2.7	1.5
ICSB 52	79	96	143	135	3.9	3.0	3.1	0.8	71.9	57.1	2.4	0.5
296 B	88	104	100	100	2.8	2.1	2.2	0.6	66.7	53.7	1.4	0.3
4111 B	80	88	167	170	3.4	2.8	3.3	1.8	74.7	74.7	2.4	1.4
185 B	78	86	180	183	3.0	3.0	3.4	1.8	75.5	73.8	2.5	1.4
Dagadi Solapure	81	91	230	243	3.3	2.9	4.8	2.3	81.4	79.8	3.9	1.8
Parbhani jyothi	86	95	267	283	3.5	3.2	3.3	0.9	76.5	68.6	2.5	0.6
Phule Anuradha	73	77	230	217	3.8	3.4	4.1	2.4	79.2	80.2	3.3	2.0
Phule vasudha	83	91	243	243	3.4	3.1	3.6	1.4	77.6	76.7	2.8	1.1
SPV 1359	82	88	261	237	3.5	3.0	3.5	1.4	79.9	74.4	2.8	1.1
SPV 1411	80	88	250	250	3.8	3.3	4.1	2.1	79.4	74.7	3.3	1.6
M35-1	79	92	207	217	3.5	3.0	3.1	1.5	81.1	77.6	2.5	1.2
Barshizoot	78	93	230	223	3.6	3.1	4.2	2.0	76.6	70.3	3.3	1.4
ICSR 149	86	96	147	150	2.3	1.8	3.1	0.9	77.4	74.4	2.4	0.7
ICSR 93034(IS 12611)	78	91	193	178	3.6	3.0	3.3	1.1	80.4	77.3	2.6	0.9
Shangavi Red	70	76	140	127	2.7	2.3	2.0	1.2	72.7	58.4	1.5	0.5

Table 4. Mean performance of 16 sorghum genotypes for studied traits in two dates of sowing (T4 and T5).

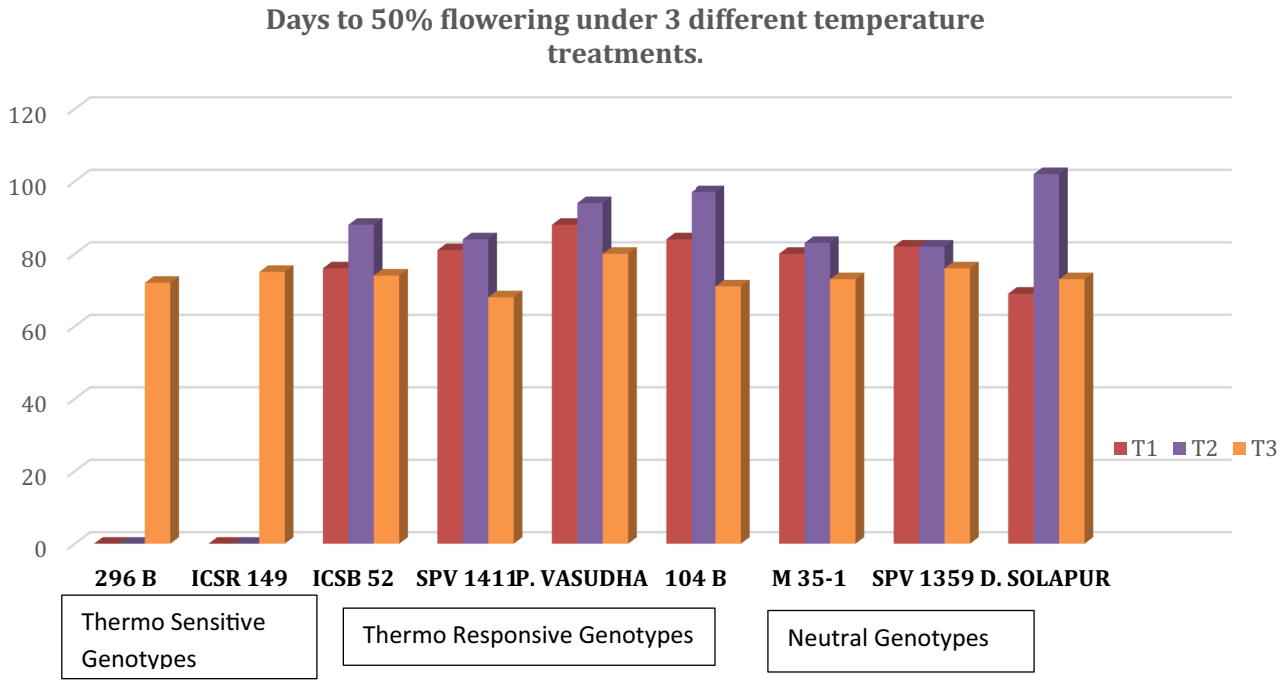


Fig. 1. Response of Sorghum genotypes to days to 50% flowering under 3 different temperature treatments.

with Shanghai Red consistently flowering earliest and Phule Vasudha or ICSR 149 flowering latest. Genotypes 296B and ICSR 149 failed to flower under both cold treatments, indicating high thermo-sensitivity. Rainy-season–adapted genotypes (104B, 185B, 4111B, and ICSB 52) exhibited limited delay under T1 but showed pronounced flowering delay under T2. In contrast, post-rainy landrace–derived genotypes (Barshi Zoot, Dagdi Solapur, M35-1, and SPV 1359) maintained stable flowering under T1, while SPV 1411 and Phule Vasudha showed moderate delay under T2. Genotypes M35-1, Phule Anuradha, Shanghai Red, and SPV 1359 were consistently stable across both cold treatments. Based on flowering response to temperature, genotypes were classified as thermo-sensitive, thermo-responsive, or temperature-neutral (Fig. 1).

For field experiment the mean flowering time for 1st DOS (T4) was 80 days ranging from 70 days for Shanghai Red and 88 days for 296B. The mean flowering time was delayed in 2nd DOS (T5) *i.e.* 90 days ranging from

76 days for Shanghai Red and 104 days for 296B (Table 4). Most of the rainy season adopted genotypes 296B, ICSB 52 and 104B showed delayed flowering in 2nd DOS compared to 1st DOS. The genotypes Phule Anuradha, SPV 1359 and SPV 1411 flowered at same time (stable) in both dates of sowings.

Days to maturity
The mean days to maturity ranged from 88 days (Shanghai Red) to 134 days (Phule Vasudha) in T1, 90 days (Shanghai Red) to 131 days (Phule Jyoti) in T2 and 75 days (Shanghai Red) to 117 days (Phule Vasudha) in T3. There is a comparatively increase in days to maturity in cold treatments compared to control. The genotype Phule Vasudha was matured last in T1 and T3 (control), whereas Parbhani Jyothi matured last in Treatment T2.

Plant height
In controlled environment experiment, mean plant height ranged from 60–143 cm in T1, 57–122 cm in T2, and 71–118 cm under control (T3). Phule Vasudha was consistently the tallest genotype across all treatments, whereas 296B was the shortest under cold treatments and ICSR 149 under control (T3). Most genotypes exhibited increased plant height under T1 compared to the (T3), while T2 showed height values largely comparable to the control (T3). Overall, cold treatment T1 promoted plant height, whereas T2 had minimal effect. For field experiment, the mean plant height ranged from 100 to 267 cm in 1st DOS (T4) and 100 cm to 283 cm in 2nd DOS (T5). The plant height was found to be stable for both the sowings. 296B was the shortest genotype with 100 cm height in both the sowings and Parbhani Jyoti was the tallest in both the sowings. In general, the post rainy season genotypes were taller than the rainy season adopted genotypes.

Panicle harvest index (PHI)
Indicator of seed set and grain filling efficiency, showed wide genotypic variation under cold stress. For control environment experiments, PHI ranged from 6–69% in T1, 3–60% in T2, and 40–73% under control conditions. Cold treatments substantially reduce PHI compared to the control, with a more pronounced reduction in T2 than T1. Genotypes 296B and ICSR 149 failed to flower under cold stress, while rainy-season–adapted genotypes 185B and ICSB 52 exhibited markedly low PHI, indicating high cold sensitivity. In contrast, Phule Anuradha, Parbhani Jyoti, Phule Vasudha, Barshi Zoot, Dagdi Solapur, M35-1, and SPV 1359 maintained relatively higher PHI across treatments. Among these, the landrace cultivars M35-1 and Dagdi Solapur showed the greatest stability across temperature regimes. For field experiment Sowing date and genotype significantly influenced PHI, with a clear reduction in the second date of sowing (T5). PHI ranged from 66.7–81.4% in the first sowing (T4) and 53.7–80.2% in the second (T5). Genotype 296B consistently showed the lowest PHI, along with ICSB 52, indicating cold sensitivity. In contrast, post-rainy–adapted genotypes Dagadi Solapur, Phule Anuradha, SPV 1411, and M35-1 maintained higher PHI across sowing dates. The decline in PHI among rainy-season genotypes with delayed sowing reflects increased reproductive-stage cold stress.

Pollen viability
Temperature stress affected pollen viability across genotypes. Mean pollen viability ranged from 85–91% in T1, 45–88% in T2, and 90–94% under control (T3). Pollen viability remained largely stable in T1 and T3, whereas a marked reduction was observed in T2. Genotypes 185B, 4111B, ICSB 52, and SPV 1411 exhibited a sharp decline in pollen viability under T2, indicating high cold sensitivity. In contrast, the remaining genotypes maintained relatively stable pollen viability under cold stress.

Grain yield
In the control environment experiment, Grain yield differed significantly among genotypes and was strongly reduced under low-temperature treatments. Mean grain yield ranged from 0.8–28.6 g plant^{-1} in T1, 0.4–17.7 g plant^{-1} in T2, and 10.8–50.9 g plant^{-1} under (T3). Genotypes 185B, ICSB 52, and Shanghai Red consistently produced low yields across treatments, whereas M35-1, Phule Anuradha, Barshi Zoot, Dagdi Solapur, and SPV 1359 maintained higher yields. Rainy-season B and R lines showed drastic yield reduction under cold stress, while post-rainy cultivars exhibited comparatively lower yield penalties. In field experiment, Grain yield was significantly influenced by genotype and sowing date, with mean yield declining from 2.64 to 1.12 kg plot^{-1} under delayed sowing (T5), reflecting reproductive-stage cold stress. Yield ranged from 1.4–3.9 kg in the first sowing (T4) and 0.3–2.0 kg in the second (T5). Dagadi Solapur and Phule Anuradha were highest yielders, while 296B, ICSB 52, ICSR 149, and ICSR 93,034 showed severe yield penalties, indicating cold sensitivity.

Seed weight
Mean seed weight showed little variation between sowing treatments (T4 and T5). The highest seed weight was recorded in Phule Anuradha and SPV 1411 under T4, with Phule Anuradha remaining highest under T5. ICSR 149 consistently recorded the lowest seed weight, followed by ICSB 52 and 296B, indicating cold sensitivity. In contrast, Parbhani Jyothi, Phule Anuradha, Phule Vasudha, SPV 1359, and SPV 1411 were least affected by delayed sowing.

Panicle weight
Panicle weight declined sharply under T5 compared to T4, ranging from 2.0–4.8 kg in T4 to 0.6–2.4 kg in T5. Dagdi Solapur produced the heaviest panicles under both treatments. Post-rainy–adapted genotypes, including Dagdi Solapur, Phule Anuradha, SPV 1411, and Barshi Zoot, maintained higher panicle weight across sowing treatments.

	Treatment 1		Treatment 2		Treatment 3		Pooled	
	PHI	PV	PHI	PV	PHI	PV	PHI	PV
PV	0.25NS		0.52NS		0.69**		0.52*	
GY	0.88**	0.07NS	0.82**	0.39NS	0.88**	0.70**	0.74**	0.33NS

Table 5. Pearson correlation analysis for Panicle harvest Index, Pollen Viability, and Grain yield for three cold treatments and polled analysis (T1 to T3).

	Treatment 4			Treatment 5		
	SW	PW	PHI	SW	PW	PHI
SW	1			1		
PW	052*	1		0.58*	1	
PHI	0.36NS	0.68**	1	0.46Ns	0.66**	1
GY	0.55*	0.99**	0.75**	0.62*	0.97**	0.78**

Table 6. Pearson correlation analysis yield contributing traits across two sowing dates (T4 and T5). SW: Seed weight; PW: panicle weight; PHI; Panicle harvest Index; GY: Grain yield.

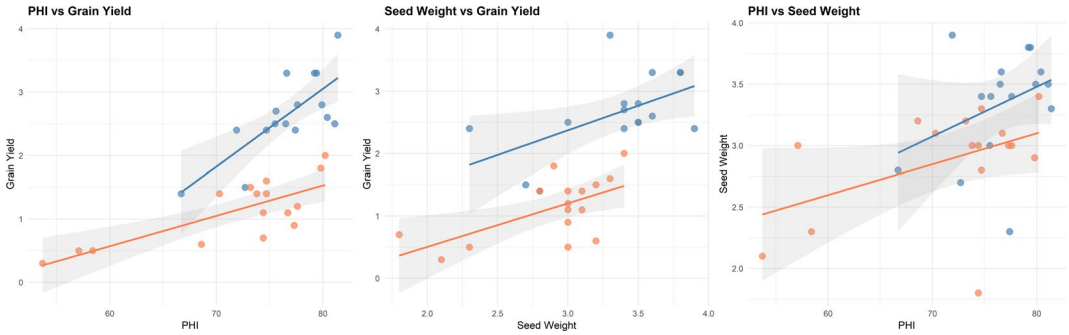


Fig. 2. Relationship of seed weight, Panicle Harvest Index and Panicle weight with grain yield in 1st and 2nd date of sowing. The r value marked with **denotes significance at $p \leq 0.01$.

Association of panicle harvest index, pollen viability and seed weight with grain yield in five different cold treatments (T1 to T5)

The association of agronomic traits studied with the grain yield was calculated in each cold treatment separately and as a pooled analysis (Table 5). The highest significant positive correlation was shown by PHI with grain yield in all three treatments (T1 $r^2 = 0.88$, T2 $r^2 = 0.82$ and T3 $r^2 = 0.88$; $P \leq 0.01$) and in pooled analysis (pooled $r^2 = 0.74$; $P \leq 0.01$). The pollen viability showed a non-significant positive correlation with grain yield in cold treatment T1, T2, and in pooled analysis, whereas it showed a significant positive correlation in T3 control ($r^2 = 0.70$, $P \leq 0.01$). There was a significant positive correlation between PHI and PV in cold treatment T3 ($r^2 = 0.69$, $P \leq 0.01$) and in pooled analysis ($r^2 = 0.52, \leq 0.01$). In the case of a field experiment, the association of seed weight, PHI and Panicle weight was studied with grain yield (Table 6). The seed weight showed a significant positive correlation with grain yield in both T4 ($r^2 = 0.55$, $p \leq 0.05$) and T5 ($r^2 = 0.62$, $p \leq 0.05$). PHI also showed significant positive correlation with grain yield, in T4 it was ($r^2 = 0.75$, $p \leq 0.01$) and T5 ($r^2 = 0.78$, $p \leq 0.01$). The panicle weight showed a strong positive association with grain yield in both the sowings ($r^2 = 0.99$, $p < 0.01$) in T4 and ($r^2 = 0.97$, $p < 0.01$) in T5. All the yield-related traits showed a significant positive association with grain yield. (Fig. 2).

Ranking of genotypes based on mean performance over three temperature treatments/ experiments using HA-GGE biplot

The Genotype ranking was conducted based on mean performance in five temperature treatments using a GGE biplot for Panicle Harvest Index (PHI) (Fig. 3a) and Grain Yield (Fig. 3b). The arrow of the Average Environment Coordination (AEC) abscissa in the biplot indicates the direction of increasing mean performance, thereby enabling the ranking of genotypes according to their performance.

For PHI, the first two principal components (PCs) explained 94.46% of the total variation. Genotype G13 (Phule Anuradha) ranked highest, followed by G11 (Phule Jyothi), G7 (D. Solapur), and G10 (M35-1). In contrast, genotypes G5 (ICSB 52), G3 (296B), G14 (Shanghai Red), and G2 (185B) exhibited poor performance for PHI. The cold stress treatments T1, T2 and T3 are grouped, suggesting similar discriminating ability for PHI, whereas treatments T4 and T5 are relatively less stressful. In the case of grain yield, the first two PCs accounted

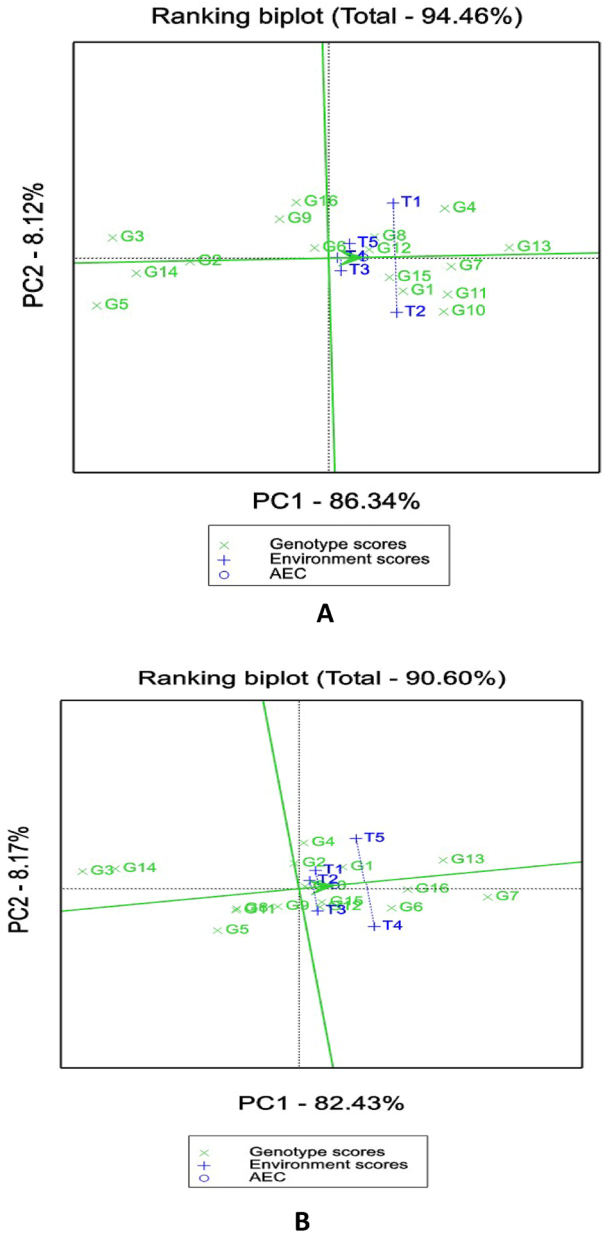


Fig. 3. (**A**) GGE ranking biplot for Panicle Harvest Index (4A), explaining stability of 16 sorghum genotypes. (**B**) GGE ranking biplot for Grain Yield 4 (**B**) explaining stability of 16 sorghum genotypes.

for 90.60% of the total variation. Genotypes positioned on the positive side of the AEC abscissa and closer to the AEC ordinate exhibited higher and more stable grain yield. Genotypes G7 (D. Solapur), G13 (Phule Anuradha), and G16 (SPV 1411) showed superior yield performance across environments, whereas genotypes located farther from the AEC axis showed greater instability. Genotypes G3 (296B) and G14 (Shanghai Red), showed poor performance in terms of grain yield. Among the cold treatments, T4 and T5 were more discriminating for grain yield, while treatments T1 to T3 cluster together, indicating a similar pattern for grain yield in low temperature stress.

Stress tolerance indices

Stress tolerance indices based on six commonly used indices: stress tolerance (TOL), mean productivity (MP), geometric mean productivity (GMP), stress susceptibility index (SSI), stress tolerance index (STI), and yield stability index (YSI) (Table 7) were calculated across three contrasting stress scenarios, namely T3-T1 (severe stress), T3–T2 (moderate stress) and T4–T5 (mild stress).

Performance under severe stress (T3 vs T1)

The severe stress condition (T1 = 10oc) clearly discriminates against the tolerant and susceptible genotypes. The genotype 4111B was found to be most tolerant based on recorded the lowest TOL (1.4), highest GMP (29.29),

a. Cold tolerance indices based on field experiment (T4 vs T5)								
Genotype	**Yp (T4)**	**Ys (T5)**	**TOL**	**MP**	**GMP**	**SSI**	**STI**	**YSI**
104 B	2.7	1.5	1.2	2.1	2.0	0.8	0.6	0.6
ICSB 52	2.4	0.5	1.9	1.5	0.6	1.4	0.2	0.2
296 B	1.4	0.3	1.1	0.9	0.2	1.4	0.1	0.2
4111 B	2.4	1.4	1.0	1.9	1.7	0.7	0.5	0.6
185 B	2.5	1.4	1.1	2.0	1.8	0.8	0.5	0.6
Dagadi Solapure	3.9	1.8	2.1	2.9	3.5	0.9	1.0	0.5
Parbhani jyothi	2.5	0.6	1.9	1.6	0.8	1.3	0.2	0.2
fPhule Anuradha	3.3	2.0	1.3	2.7	3.3	0.7	0.9	0.6
Phule vasudha	2.8	1.1	1.7	2.0	1.5	1.1	0.4	0.4
SPV 1359	2.8	1.1	1.7	2.0	1.5	1.1	0.4	0.4
SPV 1411	3.3	1.6	1.7	2.5	2.6	0.9	0.8	0.5
M35-1	2.5	9.0	1.3	1.9	1.5	0.9	0.4	0.5
Barshizoot	3.3	1.4	1.9	2.4	2.3	1.0	0.7	0.4
ICSR 149	2.4	0.7	1.7	1.6	0.8	1.2	0.2	0.3
ICSR 93034(IS 12611)	2.6	0.9	1.7	1.8	1.2	1.1	0.3	0.3
Shangavi Red	1.5	0.5	1.0	1.0	0.4	1.2	0.1	0.3
b. Cold tolerance indices based on (T3 vs T1)								
Genotype	**YP (T3)**	**YS (T1)**	**TOL**	**MP**	**GMP**	**SSI**	**STI**	**YSI**
104 B	39.8	12.9	26.9	26.35	22.65	1.09	0.4	0.32
ICSB 52	36.5	0.8	35.7	18.65	5.4	1.59	0.02	0.02
296 B	10.8	*	*	*	*	*	*	*
4111 B	30	28.6	1.4	29.3	29.29	0.08	0.66	0.95
185 B	26.6	4.8	21.8	15.7	11.3	1.33	0.1	0.18
Dagadi Solapure	30.8	19.5	11.3	25.15	24.49	0.6	0.46	0.63
Parbhani jyothi	23.6	8.8	14.8	16.2	14.4	1.01	0.16	0.37
Phule Anuradha	37.7	19.5	18.2	28.6	27.1	0.79	0.57	0.52
Phule vasudha	44.3	11.7	32.6	28	22.77	1.2	0.4	0.26
SPV 1359	42.7	18.3	24.4	30.5	27.96	0.93	0.61	0.43
SPV 1411	36	15.5	20.5	25.75	23.63	0.93	0.43	0.43
M35-1	50.9	14.5	36.4	32.7	27.17	1.17	0.57	0.28
Barshizoot	39.2	13.1	26.1	26.15	22.67	1.09	0.4	0.33
ICSR 149	33.3	*	*	*	*	*	*	*
ICSR 93034 (IS 12611)	37.9	12.4	25.5	25.15	21.67	1.1	0.36	0.33
Shangavi Red	19.4	3.1	16.3	11.25	7.75	1.38	0.05	0.16
b. Cold tolerance indices based on (T3 vs T2)								
Genotype	**YP (T3)**	**Ys (T2)**	**TOL**	**MP**	**GMP**	**SSI**	**STI**	**YSI**
104 B	39.8	17.7	22.1	28.75	26.55	0.72	0.54	0.44
ICSB 52	36.5	17.4	19.1	26.95	25.2	0.67	0.49	0.48
296 B	10.8	*	*	*	*	*	*	*
4111 B	30	10.2	19.8	20.1	17.49	0.85	0.24	0.34
185 B	26.6	1.3	25.3	13.95	5.88	1.23	0.03	0.05
Dagadi Solapure	30.8	4.7	26.1	17.75	12.03	1.11	0.11	0.15
Parbhani jyothi	23.6	11.3	12.3	17.45	16.33	0.67	0.21	0.48
Phule Anuradha	37.7	9.5	28.2	23.6	18.94	0.98	0.28	0.25
Phule vasudha	44.3	7.3	37	25.8	17.98	1.07	0.25	0.16
SPV 1359	42.7	4.8	37.9	23.75	14.32	1.15	0.16	0.11
SPV 1411	36	7.7	28.3	21.85	16.65	1.01	0.21	0.21
Continued								

M35-1	50.9	12.7	38.2	31.8	25.43	0.97	0.5	0.25
Barshizoot	39.2	0.4	38.8	19.8	3.96	1.29	0.01	0.01
ICSR 149	33.3	*	*	*	*	*	*	*
ICSR 93034 (IS 12611)	37.9	1.8	36.1	19.85	8.26	1.24	0.05	0.05
Shangavi Red	19.4	2	17.4	10.7	6.23	1.16	0.03	0.1

Table 7. Cold Tolerance indices of 16 sorghum genotypes between different treatment combinations. YP: Yield under non stress condition; YSI: Yield under cold stress condition; MP Mean Productivity; GMP: Geometric Mean productivity; SSI: Stress Suceptibility Index; STI: Stress tolerance Index; YSI: Yield Stability Index.

highest STI (0.66), and maximum YSI (0.95), indicating strong stability of these genotypes for cold stress. Similarly, the genotypes Dagdi Solapur and Parbhani Jyoti showed moderate tolerance based on intermediate STI (0.46 and 0.16, respectively) with acceptable YSI values. Conversely, the genotypes ICSB 52, Shanghai Red and 185 B were found to be highly susceptible as evidence from high SSI (> 1.3), extremely low STI (< 0.1), and poor YSI (< 0.2).

Performance under moderate stress (T3 vs T2)
The genotype 104 B recorded the highest STI value (0.54) along with high mean GMP (26.55), indicating consistent yield performance. The genotype M-35 -1 also performed better with high MP (31.8), GMP (25.43), and STI (0.50), reflecting broad adaptability. Parbhani Jyoti with relatively low SSI (0.67) and high YSI (0.48) demonstrates better yield stability despite moderate yield potential. In contrast , the genotypes Barshi zoot and ICSR 93,034 (IS 12,611) showed extremely high SSI (> 1.2), and very low STI (≤ 0.05), identifying them as highly stress-susceptible genotypes.

Stress tolerance indices based on field experiment performance under mild stress (T4 vs T5)
The mean productivity (MP) representing mean productivity in both conditions was highest for genotype Dagdi Solapur (2.9), followed by Phule Anuradha (2.7), SPV 1411 (2.5) and Barshi Zoot (2.4). The highest Geometric Mean productivity was observed for Dagdi Solapur (3.5) followed by Phule Anuradha (3.3). Stress Susceptibility Index (SSI) is an indication of the vulnerability of genotypes and yield loss. The lowest SSI was recorded for genotype Phule Anuradha and 4111B (0.7), followed by 104B & 185 B (0.8) and the highest SSI was recorded by genotype ICSB 52 & 296 B (1.4). In case of stress tolerance index (STI), the STI values ranged from 0.1 (296B & Shanghai Red) to 1.0 (Dagdi Solapur), indicating ample variation in stress tolerance in the study genotypes. genotypes with high STI values viz., Dagdi Solapur (0.9), Phule Anuradha (0.9) and SPV 1411(0.8), suggesting better performance in both conditions, while genotypes with low STI viz., 296 B, Shanghai red, ICSR 149 and ICSB 52 showed poor adaptability to stress conditions. In the case of the Yield Stability Index (YSI) measure of yield retained (stability) under stress conditions to non-stress conditions, the YSI ranged from 0.2 (Parbhani Jyoti) to 0.6 (104 B, 4111 B, 185B and Phule Anuradha), the higher YSI genotypes indicated better stability under stress conditions.

The comparative analysis of Stress tolerance indices using three different scenarios, the genotypes Phule Anuradha, SPV 1359, M 35–1 and Dagdi Solapur showed consistent robustness with a favourable combination of high MP, GMP, and STI along with moderate to low SSI values. The genotypes ICSB 52 and 185 B were consistently susceptible across stress-prone environments.

Discussion

The central part of India *i.e.* Maharashtra, Northern Karnataka and Telangana, grows sorghum in large areas during postrainy (*rabi*) season. Three million tons of sorghum is produced from 5–7 million ha. of cropping in postrainy sorghum[15]. The crop is grown under residual soil moisture, and to avoid critical terminal drought and the crop is sown before the last week of October, which exposes the flowering phase to cold temperatures in December –January. The sowing cannot be advanced as early sown crop is affected by shoot fly and secondly, the photoperiod sensitive genotypes don't flower even if we sow earlier[16]. Most of the postrainy sorghums come to flowering around Christmas time, which coincides with severe cold[16]. It is difficult to assess the cold tolerance in field experiments as there will be confounding effects by other abiotic stresses. Genotypic cold tolerance during the vegetative stage is reported to be independent of cold tolerance during the reproductive stage[14]. Consequently, the identification of the best phenotypic indicator for selecting cold-tolerant genotypes at the flowering stage can lead to the identification of cold-tolerant lines. In the present study, it was observed that the panicle harvest index (PHI) could serve as a reliable indicator of reproductive stage cold tolerance in sorghum. The effects of cold temperatures on days to 50% flowering, panicle harvest index, and other yield components are discussed herein.

Days to 50% flowering

In a controlled environment experiment, cold stress resulted in delayed flowering and in severe cold conditions, the genotypes failed to flower. In the present study, genotypes 296B and ICSR 149 were unable to flower in cold treatments T1 and T2, which indicated that these are highly susceptible genotypes for cold stress. Longer delays in flowering were shown mostly by rainy season-adapted genotypes like 104B, ICSB 52, compared to typical postrainy-adapted land race cultivars. This clearly indicated that the cultivars developed for the rainy season, like B lines and R lines, were more susceptible to cold, which may be the reason for the low yield of rainy season-

adapted hybrids when grown in the post-rainy season. The postrainy season ruling genotypes M 35–1, Barshi zoot, Dagdi Solapur being photoperiod sensitive and cold tolerant were having ability to flowering same time, this is confirmed the finding by[17]. The photoperiod sensitivity and thermal insensitivity of M-35–1 have resulted in its widespread utilisation as a parent in the development of numerous successful improved progenies suitable for adoption during the post-rainy season[18]. There is an urgent need to replace the popular 296B line with improved cold-tolerant B lines to enable the rainy season hybrids to be grown in the post-rainy season. Similar results for DTF were observed[19]. The field experiment of two dates of sowings also indicated the effect of cold temperature on flowering of genotypes. The mean days to 50% flowering was delayed in 2nd DOS (T5) as the peak booting stage coincided with cold temperature. The delay in flowering observed in genotypes adopted for the rainy season indicates their sensitivity to cold temperatures, whereas genotypes adopted for the post-rainy season exhibit comparatively less delay in flowering despite cold temperatures. Maqbool et al.[20] reported that even inherently tolerant genotypes can suffer from cold stress. During flowering, low temperature stress can result in delayed panicle emergence and heading in sorghum. This study suggests that precision can be achieved by synchronising the stages of flowering to a narrow window of calendar days through staged sowing. This approach ensures that the flowering time of genotypes aligns with prevailing low temperatures, which also aids in understanding the genetics of photoperiod sensitivity and thermal sensitivity[21].

Panicle harvest index (PHI)

The panicle harvest index is ratio of grain mass to panicle mass and successfully explains the effect of cold stress on the genotypes in the present study. The higher PHI means most of the florets are able to convert into grains. The cold and heat are known to adversely affect the reproductive growth in many cereals. The lowest PHI was observed in the T2 i.e. 3%, followed by T1 = 6%. Reductions of these values in cold treatment were an indication in failure of reproductive growth or failure of grain to fill its potential. The high positive correlation of PHI to grain yield in individual cold treatment and in pooled analysis indicated that the PHI is best indicator for selecting cold tolerant genotypes.

The rainy season adopted B lines and R lines found in group of low PHI and most post rainy season genotypes showed higher PHI in all the treatments. Moreover, Barshi Zoot, Dagadi Solapur, M35-1 and SPV1359 were ranked as stable genotype by GGE biplots. This finding corroborates the earlier discovery by Krishnamurty et al.[19] that the majority of cold-tolerant genotypes originated from MPKV-Rahuri, including M 35–1, Dagadi Solapur, Barshi Zoot, and SPV 1359 are stable performers, as the Western Maharashtra Agro-ecology is ideally suited for cultivating postrainy sorghum. In these areas, the days are typically warmer and brighter, but minimum temperatures are known to drop below 10 °C for the last 7 weekends and below 5 °C for at least a week during winter, indicating moderate cold conditions in postrainy sorghum regions[22]. Varieties developed in these environments demonstrate greater adaptation to cold stress, making these locations potential hotspots for screening cold-tolerant breeding materials[19].

In a field experiment, PHI was found to be reduced with 2nd DOS (T5) for most of the genotypes, whereas genotypes like Dagdi Solapur, Phule Anuradha and Phule Vasudha were found stable in both sowings. The highest PHI shown by Dagdi Solapur (81.4%) in 1st DOS (T4), PHI is also a threshing index and represents the portion of grain yield per whole panicle. The cold is the main factor that affects reproductive growth, and measurement of the PHI is the main screening factor. A good level of genetic discrimination was observed in the genotypes used in this study. PHI is closely related to spikelet fertility in sorghum[23], in rice[5,7] and in wheat[24]. The positive association of PHI and grain yield indicated that recording PHI in yield evaluation trials will simultaneously select for grain yield and cold tolerance. The results of the field study are also confirmed by the control environment studies. In both experiments, the post-rainy season adopted genotypes like Phuke Anuradha, Dagadi Solapur, M 35–1, SPV 1359 and SPV 1411 showed higher values of PHI hence, these genotypes should be used in the breeding program for the improvement of cold tolerance in sorghum.

Yield components

The yield is the most important component in any breeding program. In the present study, the effect of cold temperature on yield is noted using grain yield/plant and pollen viability in a controlled environment experiment, and seed weight, panicle weight/plot and grain yield /plot in a field experiment. Low temperature reduces the yield of sorghum through pollen sterility during panicle development by 20%, with an increase in floret abortion with shrivelled anthers and withering stigmas by 23% and a reduction in the number of seeds by 39% was reported earlier[11,23]. Thakur et al.[9] also reported that low temperature stress at the reproductive stage causes flower abscission, pollen sterility, pollen tube destitution, ovule abortion and poor seed set.

In a controlled environment study, cold treatment (T2) showed a drastic reduction in pollen viability compared to the control which ultimately affects grain yield in T2 compared to the control. The genotypes M 35–1, Dagadi Solapur, Phule Vasudha, Phule Anuradha and SPV 1359 showed high pollen viability and grain yield in all the cold treatments.

In the field experiment, Panicle weight and Grain Yield showed a drastic reduction in 2nd DOS. Whereas seed weight does not show much variation between two dates of sowing. The panicle weight also showed a high positive correlation with grain yield in both sowings. After fertilization, the partitioning of the photosynthates from source to sink is determined by the genetic response to cold stress. This genetic variation will be calculated using panicle weight, PHI and Seed weight. The genotypes Dagadi Solapur showed the highest panicle weight in both DOS followed by Phule Anuradha and SPV 1411. The genotypes 296B and ICSB 52 were sensitive to cold, with lower panicle weight and grain yield.

Cold tolerance indices across different treatment scenarios

With the combination of three different treatment scenario, severe stress (T3 Vs T1), moderate stress (T3 Vs T1) and mild stress (field screening) (T4 Vs T5) and six stress tolerance indices together provided a comprehensive analysis of genotypes adopted to cold tolerance. The variable expression of stress tolerance indices across three different scenarios indicates cold tolerance is a quantitative trait, and reliable identification of cold tolerance genotypes requires evaluation under multiple levels of stress rather than a single stress treatment.

Among the different stress tolerance indices the indices, particularly GMP, STI & YSI were more consistent that TOL & MP for discriminating cold tolerance genotypes, this is in agreement with earlier report of Fernadez 1992). The genotypes Phule Anuradha, SPV 1359, M 35–1 and Dagdi Solapur with consistently high GMP & STI values coupled with moderate to low SSI showed stable.

performance across the cold stress treatments. The consistent stability of the genotype suggested the presence of a robust physiological mechanism governing cold stress tolerance, rather than an environment-specific response. These genotypes are superior in breeding programs to deal with unpredictable stress conditions. In contrast, genotypes ICSB 52 and 185B consistently high SSI and low STI & YSI across three comparisons, including high vulnerable to cold stress. The genotype 296B and ICSR 149 are unable to flower and produce gain in stress and moderate stress cold environments, indicating these are the highly susceptible genotypes for cold stress. The shift in genotype ranking with increasing stress intensity emphasizes that mild stress conditions tend to mask susceptibility while moderate & severe stress conditions truly revel tolerance. This is in alignment with the previous report of Blum (2011)[25] that moderate stress environments are optimal for selection as they balance discrimination power with biological factors.

The consistent strong concordance of GMP, SIT and YSI across the cold stress scenarios highlights their robustness and selection criteria for selecting cold tolerance genotypes and donor identification in plant breeding program.

Association analysis in different cold treatments

In the control environment treatments (T1 to T3), the strong positive association between PHI and grain yield across the cold treatments, along with pooled analysis, indicated PHI as a robust indicator for cold stress tolerance at the reproductive stage. Similar association of harvest Index with grain yield in abiotic stress condition in sorghum and other cereals were reported by (Blum., 2011., Prasad et al., 2015)where cold stress primary limits assimilated portioning and grain filling rather than biomass production, Only in T3 (control) the significant association of Pollen viability (PV) with grain yield was recorded, suggesting that PV is yield limiting factor only in optimal environment for cold stress the downward processes such as successful fertilization and grain filling (as reflect by PHI) plays dominant role. The results of correlation analysis in field experiments (T4 & T5) are further in consistent with the finding, where PHI and Panicle Weight were strongly positively associated with grain yield in both the treatments (T4 and T5). The drastic yield decline in late sowing (T5) indicates an increase in cold sensitivity during the reproductive stage, consistent with the earlier report in post-rainy sorghum by[18,19].

Ranking of genotypes based HA-GGE biplot

HA-GGE biplot effectively discriminated the genotypes based on PHI and grain yield across the treatments (T1 -T5). The genotypes such as Phule Anuradha, Phule Jyoti, Dagdi Solapur and M35-1 consistently ranked superior for PHI, highlighting the stable reproductive efficiency in cold stress. These genotypes are the best donors for cold tolerance breeding in sorghum. For grain yield M35-1 followed by Phule Anuradha and Dagdi Solapur are the most stable and high-performing genotypes; similar findings are reported for these genotypes by[18,26] . The B lines 296 B and ICSB 52 are poor performing for cold tolerance, similar to the poor performance of these B lines.

Conclusion

The traits studied provided a good level of genotypic discrimination among the 16 sorghum genotypes in both the controlled environment and the field experiment. Among these, Panicle Harvest Index was the best phenotypic indicator, which is confirmed by the results of cold treatments during the flowering stage. Its strong association with grain yield under cold stress and it is a simple and cost-effective protocol compared to the pollen viability test. The findings clearly distinguished that the postrainy-season adopted genotypes Phule Anuradha, Dagadi Solapur, M 35–1, and SPV 1359 were cold-tolerant across the environments, whereas Genotype 296B and ICSR 149 were highly susceptible to cold stress and showed delayed flowering, poor PHI, and reduced yield. The identified cold-tolerant genotypes should be used in breeding programs to improve the cold tolerance in postrainy sorghum. Their incorporation into breeding programs can contribute significantly to the development of stable, high-yielding sorghum cultivars suited to low-temperature stress environments typical of the postrainy season.

Materials and methods

Experimental material and Controlled environment phenotyping

The experimental material comprised 16 sorghum genotypes, including nine released varieties and seven parental lines (Table 8). All genotypes were obtained from the sorghum breeding program at ICRISAT Hyderabad, India. These genotypes are well-adapted to post-rainy season cultivation and known to flower between 65–80 days after sowing. The study was conducted to evaluate the response of these genotypes under control (normal temperature) and cold stress conditions using a controlled environment phenotyping approach.

Serial No	Genotypes	Main characteristics	Adapted season	Sources
1	104 B	High yielding, B line	Rainy	
2	ICSB 52	High yielding, B line	Rainy	ICRISAT, India
3	296 B	High yielding, B line	Rainy	DSR, India
4	4111 B	High yielding, B line	Rainy	ICRISAT, India
5	185 B	High yielding, B line	Rainy	MPKV, India
6	Dagdi Solapur	Bold lustrous, High yielding	Post- rainy	MPKV, India
7	Parbhani Jyoti	High yielding	Post- rainy	VNMKV, India
8	Phule Anuradha	High yielding	Post- rainy	MPKV, India
9	Phule Vasudha	High yielding	Post- rainy	MPKV, India
10	SPV 1359	High yielding	Post- rainy	MPKV, India
11	SPV 1411	High yielding	Post- rainy	VNMKV, India
12	M 35–1	Bold lustrous, High yielding	Post- rainy	Mohal, India
13	Barshi zoot	High yielding	Post- rainy	Barshi, India
14	ICSR 149	High yielding, R line	Rainy	ICRISAT, India
15	ICSR 93034	High yielding, R line	Rainy	ICRISAT, India
16	Shanghai red	-	-	China

Table 8. Characteristics of sorghum genotypes evaluated for cold tolerance at different cold treatment at ICRISAT, Patancheru.

Experimental design and planting

Controlled environment experiment and experimental design

The experiment was conducted at the Controlled Environment Research Facility at ICRISAT, India, following a Randomised Complete Block Design (RCBD) with three replications. The experiment consisted of three temperature treatments, representing control and stress conditions.

- T1 – Moderate Cold Stress: Day/Night temperature of 25 °C / 10 °C
- T2 – Severe Cold Stress: Day/Night temperature of 25 °C / 5 °C
- T3 – Control (Normal Condition): Natural greenhouse environment with temperatures ranging from 22–36

Each genotype was evaluated under all three treatments, and each treatment was replicated three times, ensuring a reliable comparison of genotype performance under control and stress environments.

Based on long-term average minimum temperatures during the post-rainy sorghum-growing season, planting was scheduled to ensure that plants were exposed to the designated temperature regimes during the sensitive reproductive stage. Genotype-specific responses to temperature treatments are presented in Fig. 4 .

Planting and growth conditions

Potting A total of 144 poly-trainer pots (8-inch) were used. Pots were filled with a 2:1:1 mixture of soil, farm-yard manure (FYM), and vermicompost.

Sowing Three seeds per pot were sown and later thinned to one healthy plant per pot after emergence.

Nutrient management Urea @ 10 g per pot was applied at 25 and 50 days after sowing (DAS).

Water management Pots were irrigated regularly to maintain optimal soil moisture throughout the experiment.

Cold stress application

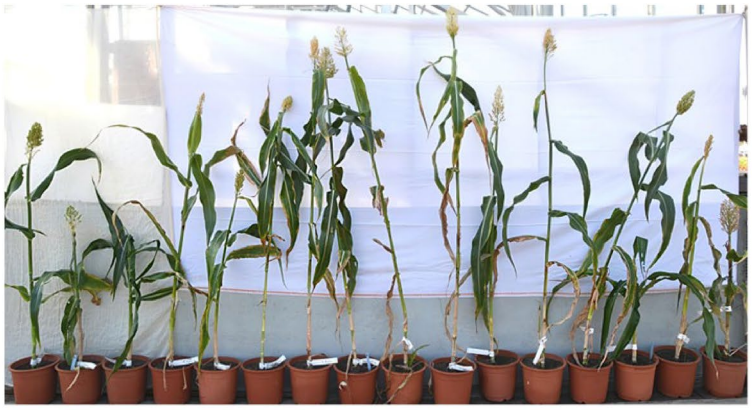
Initially, all plants were grown under ambient conditions (Fig. 5). After 50 days of planting, the plants were moved to growth chambers to expose them to cold stress, as indicated in T1 and T2 treatments. Cold stress treatment was given for 30 days. Once the cold stress treatment was completed, the plants were returned to normal temperatures in the greenhouse.

Data collection

Data were recorded on traits such as plant height, days to 50% flowering, days to maturity, grain weight g plant^{-1}, panicle harvest index, and pollen viability percentage. Panicles were harvested separately to measure panicle weight before threshing and grain weight after threshing. The panicle harvest index (PHI) was calculated as the ratio of grain weight to panicle weight.

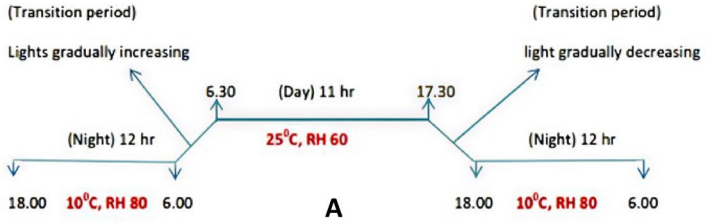
Pollen viability test

During anthesis, unopened anthers were collected in the morning using dissecting forceps, a scalpel, and a needle. The anthers were then opened to extract the pollen, which was subsequently transferred onto a microscopic glass slide with a drop of aceto-carmine stain (HIMEDIA GRM 4942). Mature anthers were crushed, and the pollen



1) **Treatment 1**

Day = Temp 25°C, Relative Humidity 60
Night = Temp 10°C, Relative humidity 80

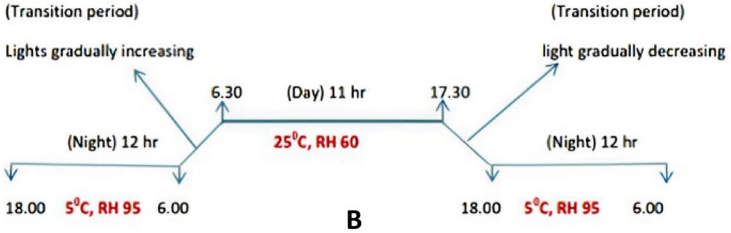


A



2) **Treatment 2**

Day= Temp 25°C, Relative humidity 60
Night = temp 5°C, Relative Humidity 95



B

Fig. 4. Cold Tolerance treatments profile. (**A**) Cold tolerance Treatment-1 (25 °C Day time /10 °C in night time) profile showing the temperature regime and duration of cold stress applied to test genotypes. (**B**) Cold tolerance Treatment-2 (25 °C Day time /5 °C in night time) profile showing the temperature regime and duration of cold stress applied to test genotypes. (**C**) Cold tolerance Treatment-3 (22–36 °C) minimum and maximum temperature regime applied to test genotypes.

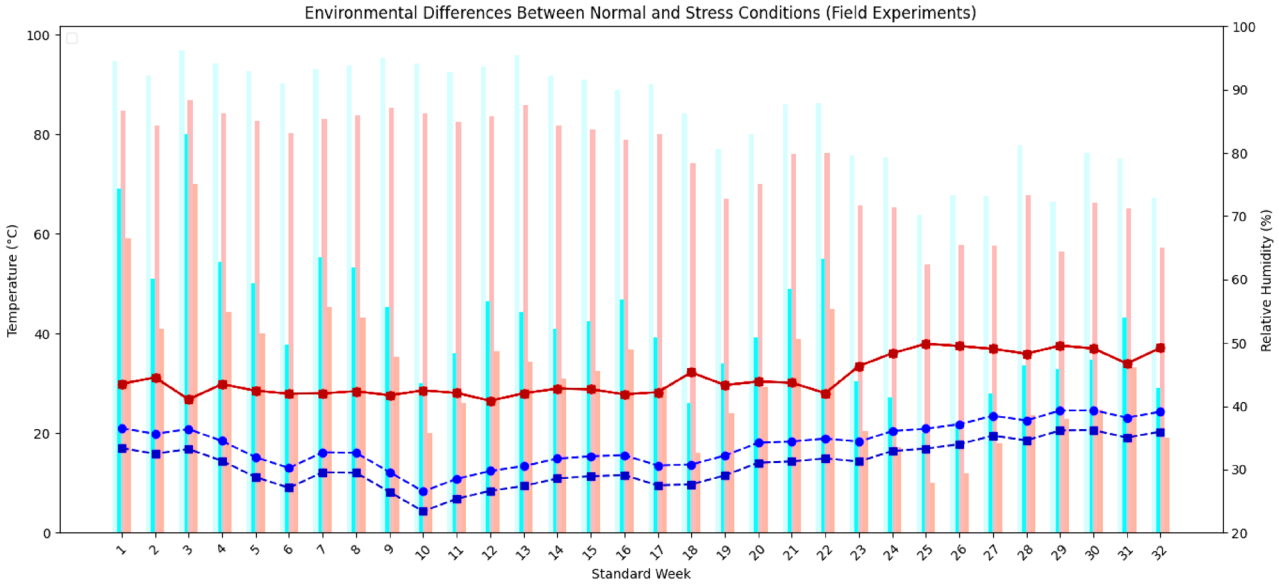


Fig. 5. Maximum and minimum temperatures in controlled environment (T3).

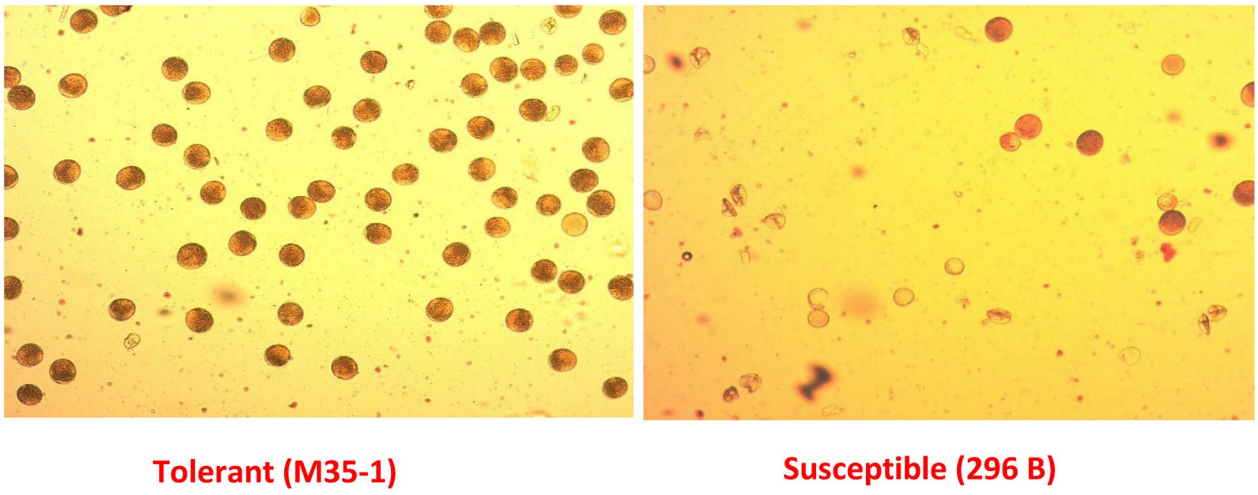


Fig. 6. Pollen Viability test conforming tolerance of M 35–1 and susceptibility of 296B to cold temperatures.

grains were stained with aceto-carmine. Cover slips were gently placed on the slides, with five slides prepared for each genotype. For each slide, ten randomly selected microscopic fields were observed under a 10 × objective (100 × magnification). Pollen viability was determined by recording darkly stained pollen grains as viable, while unstained or lightly stained grains were considered non-viable (Fig. 6). Pollen viability percentage was calculated using the following formula:

$$Pollen\ viability\% = \frac{No\ of\ dark\ stain\ viable\ pollen}{Total\ no.\ of\ pollen} \times 100$$

Field experiment

Location and design

The field experiment was conducted at ICRISAT-Patancheru (17°30′N; 78°16′E; 549 m altitude) during the post-rainy season of 2013–14, using the same 16 genotypes under RCBD with 3 replications.

Sowing and plot layout

Sowing occurred on two dates: October 29th (first date) and November 8th (second date) during the 2013–14 post-rainy season. Each experimental plot consisted of two rows measuring 2 m in length, with an inter-row spacing of 0.75 m and an intra-row spacing of 0.15 m for both sowing dates. The staggered sowing was implemented to expose the genotypes to varying thermal regimes. In the first date of sowing (DOS1), the

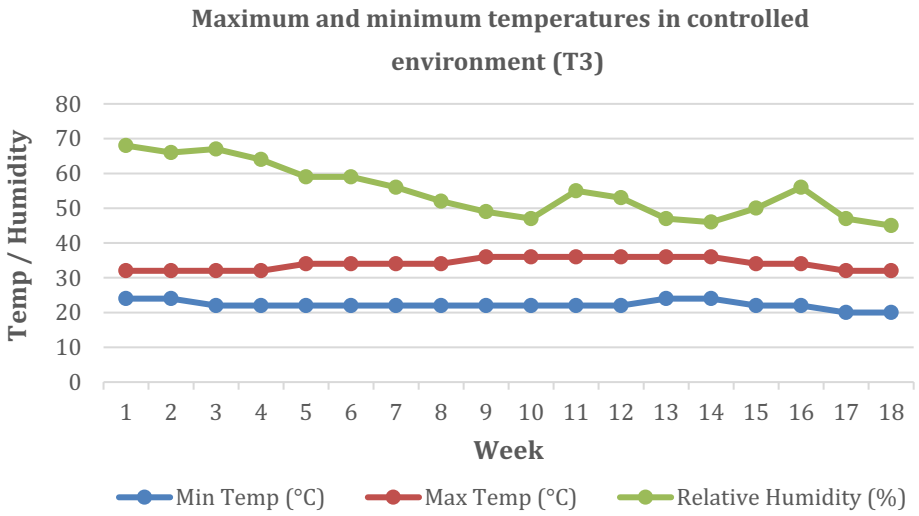


Fig. 7. Maximum and minimum temperatures in controlled and stress environment in field condition.

recorded minimum and maximum temperatures were 6.8 °C and 30.5 °C, respectively. In the second date of sowing (DOS2), the minimum and maximum temperatures were 8.6 °C and 32.4 °C, respectively (Fig. 7), allowing assessment of genotype performance under relatively cooler conditions typical of reproductive stage exposure to low temperature stress.

Crop management

To protect against shoot fly damage, seeds were treated with 70% water-soluble imidacloprid (Sensex® GSP Crop Science Pvt Ltd). Fertilisation included the surface application and incorporation of 40 kg N ha-1 and 40 kg P ha -1 as basal, with an additional 40 kg N ha-1 applied at 30 days as top dressing. Optimal irrigation was provided as needed throughout the trials. Data collection included recording the days to 50% flowering, plant height, grain weight in the whole plot, and panicle harvest index (PHI) for five randomly selected panicles from each genotype. Panicles were harvested separately, and their weights were recorded before and after threshing to calculate the PHI.

$$PHI = \frac{Total\ grain\ weight\ of\ panicle}{Panicle\ dry\ weight}$$

Statistical analysis

For the control environment experiment

The mean values of the data recorded for each plant across three replications for all traits were computed and utilised for statistical analysis. Analysis of variance (ANOVA) was conducted using a randomised complete block design (RCBD) with two factors to evaluate the effects of genotype, temperature (cold stress) treatments, and genotype × temperature treatment interactions. Additionally, correlation analysis was performed on different treatments, with yield as the dependent variable and yield components, plant height, days to 50% flowering, days to maturity, panicle harvest index, and pollen viability percentage as independent variables.

For field experiment

The aggregated mean values of data recorded on sample plants across three replications for agronomic traits were utilised for statistical analysis. All statistical analyses were conducted using GENESTAT 14th version. An analysis of variance for a randomized complete block design (RCBD) was employed to assess the effects of genotypes, dates of sowing (DOS), and genotype × DOS interaction. The Bartlett test was used to assess the homogeneity of error variance prior to pooling the two-sowing data. Analysis and estimation of stress tolerance indices: Cold tolerance indices viz., Mean productivity (MP), Tolerance index (TI), Yield stability index (YSI), Stress tolerance index (STI) and Stress Susceptibility index (SSI) for each genotype were calculated[27–29] .

The Yield Stability Index (YSI) is defined as the ratio of yield under stress conditions to yield under non-stress conditions. It serves as a measure of genotype stability across varying environments. A higher YSI value signifies a more stable genotype that performs consistently across different conditions. The Stress Tolerance Index (STI) measures the ability of a genotype to perform better in both stress and optimal conditions, emphasising high yield in both conditions. The Stress Susceptibility Index (SSI), evaluates the extent to which a genotype is affected by stress, indicating its vulnerability and associated yield loss. Lower SSI values represent genotypes that are more tolerant of stress. Mean Productivity (MP), widely used to evaluate the average yield of genotypes under both stress and non-stress conditions, favors genotypes with high mean yields and low standard deviation.

Geometric Mean Productivity (GMP) assesses the relative performance of genotypes across stress and non-stress environments and provides insight into their overall productivity under variable conditions.

Formulas used
Mean productivity (MP)

$$\mathrm{MP} = \frac{Ys + Yp}{2}$$

- YsY_sYs: Yield under stress
- YpY_pYp: Yield under non-stress (optimal) conditions

Tolerance index (TOL)

$$\mathrm{TOL} = \mathrm{Y}_p - \mathrm{Y}_s$$

- Measures the reduction in yield due to stress
- Lower TOL = greater stress tolerance

Yield stability index (YSI)

$$\mathrm{YSI} = \frac{Ys}{\mathrm{Yp}}$$

- Values closer to 1 indicate better stability under stress

Stress tolerance index (STI)

$$\mathrm{STI} = \frac{Ys\mathrm{x}Yp}{\mathrm{Yp}^{-2}}$$

$\overline{Yp}$: Mean yield of all genotypes under non-stress.

- High STI indicates high yield under both stress and non-stress

Stress susceptibility index (SSI)

- $\mathrm{SSI} = \frac{1-(Ys/Yp)}{S}$ Where $\mathrm{S} = 1 - \left(\frac{\overline{ys}}{\overline{yp}}\right)\overline{Ys}$: Mean yield of all genotypes under stress
- $\overline{Yp}$: Mean yield of all genotypes under non-stress
- Lower SSI = higher tolerance to stress

Geometric mean productivity (GMP)

$$\mathrm{GMP} = \sqrt{Ys + Yns}$$

- Ys: Yield under stress condition
- Yns: Yield under non-stress (normal) conditions

Data availability

The Authors confirm that the data supporting the findings of this study are available within the article. Raw data that support the findings of this study are available from the corresponding author, upon reasonable request.

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References

1. FAO. FAOSTAT Statistical Database. Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/en/ (2021).
2. Sajjanar, G. M., Biradar, B. D. & Biradar, S. S. Evaluation of crosses involving rabi landraces of sorghum for productivity traits. *Karnataka J. Agric. Sci.* **24**, 227–229 (2011).
3. Bavi, K., Kholdebarin, B. & Moradshahi, A. Effect of cadmium on growth, protein content and peroxidase activity in pea plants. *Pak. J. Bot.* **43**, 1467–1470 (2011).

4. Kebede, M. M., Zaman-Allah, M. & Tarekegn, A. Sorghum adaptation to low temperature stress: Recent advances and future perspectives. *Front. Plant Sci.* **14**, 1123456. https://doi.org/10.3389/fpls.2023.1123456 (2023).
5. Pereira da Cruz, R., Milach, S. C. K. & Federizzi, L. C. Rice cold tolerance at the reproductive stage in a controlled environment. *Sci. Agric.* **63**, 255–261 (2006).
6. Knoll, J., Gunaratna, N. & Ejeta, G. QTL analysis of early-season cold tolerance in sorghum. *Theor. Appl. Genet.* **116**, 577–587. https://doi.org/10.1007/s00122-007-0692-0 (2008).
7. Lafitte, H. R. et al. Improvement of rice drought tolerance through backcross breeding: Evaluation of donors and selection in drought nurseries. *Field Crops Res.* **97**, 77–86. https://doi.org/10.1016/j.fcr.2005.08.017 (2006).
8. Zinn, K. E., Tunc-Ozdemir, M. & Harper, J. F. Temperature stress and plant sexual reproduction: Uncovering the weakest links. *J. Exp. Bot.* **61**, 1959–1968. https://doi.org/10.1093/jxb/erq053 (2010).
9. Thakur, P., Kumar, S., Malik, A. J., Berger, D. J. & Nayyar, H. Cold stress effects on reproductive development in grain crops: An overview. *Environ. Exp. Bot.* **67**, 429–443. https://doi.org/10.1016/j.envexpbot.2009.09.004 (2010).
10. Zhang, X., Wang, M., Liu, Y. & Wang, Y. Understanding sorghum responses to abiotic stresses: A comprehensive review. *Plants* **12**(4), 821. https://doi.org/10.3390/plants12040821 (2023).
11. Dhopte, A. M. & Eastin, J. D. Influence of night temperature during floret differentiation on microsporogenesis and seedsetting in sorghum (Sorghum bicolor (L.) Moench). In Role of Biotech in Agriculture (eds. Prasad, B. N., Ghimire, G. P. S. & Agarwal, V. P.) 193–200 (1992).
12. Yu, J., Tuinstra, M. R., Claassen, M. M., Gordon, W. B. & Witt, M. D. Analysis of cold tolerance in sorghum under controlled environment conditions. *Field Crops Res.* **85**, 21–30. https://doi.org/10.1016/S0378-4290(03)00125-4 (2004).
13. Fischer, R. A. & Maurer, R. Drought resistance in spring wheat cultivars. I. Grain yield responses. *Aust. J. Agric. Res.* **29**, 897–912. https://doi.org/10.1071/AR9780897 (1978).
14. Singh, S. P. Use of genetic male sterility for simultaneous conversion and improvement of alien sorghums. *Crop Sci.* **17**, 482–484. https://doi.org/10.2135/cropsci1977.0011183X001700030037 (1977).
15. Kholová, J., McLean, G., Vadez, V., Craufurd, P. & Hammer, G. L. Drought stress characterization of post-rainy season (Rabi) sorghum in India. *Field Crops Res.* **141**, 38–46. https://doi.org/10.1016/j.fcr.2012.10.020 (2013).
16. Reddy, B. V. S. et al. Postrainy season sorghum: Constraints and breeding approaches. J. SAT Agric. Res. **10**, (2012).
17. Reddy, B. V. S., Seetharama, N. & House, L. R. Sorghums in the post-rainy season. I. Effect of irrigation and date of sowing on the grain and stover yields of diverse cultivars. *Exp. Agric.* **24**, 31–36. https://doi.org/10.1017/S0014479700015672 (1987).
18. Reddy, B. V. S., Ramesh, S. & Reddy, P. S. Genetic enhancement for drought tolerance in sorghum. *Plant Breed. Rev.* **31**, 189–222 (2009).
19. Krishnamurthy, L., Dinakaran, E., Kumar, A. A. & Reddy, B. V. S. Field technique and traits to assess reproductive stage cold tolerance in sorghum (*Sorghum bicolor* (L.) Moench). *Plant Prod. Sci.* **17**, 218–227. https://doi.org/10.1626/pps.17.218 (2014).
20. Maqbool, A., Shafiq, S. & Lake, L. Radiant frost tolerance in pulse crops – A review. *Euphytica* **172**, 1–12. https://doi.org/10.1007/s10681-009-0031-4 (2010).
21. Tarumoto, I., Yanase, M., Kadowaki, H., Yamada, T. & Kasuga, S. Inheritance of photoperiod-sensitivity genes controlling flower initiation in sorghum *Sorghum bicolor* Moench. *Grassl. Sci.* **51**, 55–61. https://doi.org/10.1111/j.1744-697X.2005.00009.x (2005).
22. Jahagirdar, J. E. & Borikar, S. T. Winter season adaptation features in sorghum. *Int. Sorghum Millets Newsl.* **43**, 45–46 (2002).
23. Dhopte, A. M. & Eastin, J. D. Response of sorghum to elevated night temperature imposed during floret differentiation under field conditions. *Proc. Int. Cong. Plant Physiol.* **1**, 929–934 (1990).
24. Subedi, K. D., Gregory, P. J., Summerfield, R. J. & Gooding, M. J. Cold temperatures and boron deficiency caused grain set failure in spring wheat (*Triticum aestivum* L.). *Field Crops Res.* **57**, 277–288. https://doi.org/10.1016/S0378-4290(97)00148-2 (1998).
25. Blum, A. Drought resistance–Is it really a complex trait?. *Funct. Plant Biol.* **38**(10), 753–757 (2011).
26. Patil, S. L., Sheelavantar, M. N. & Lamani, V. K. Correlation analysis among growth and yield components of winter sorghum. *I S M N* **44**, 14–17 (2009).
27. Bouslama, M. & Schapaugh, W. T. Stress tolerance in soybean. I. Evaluation of three screening techniques for heat and drought tolerance. *Crop Sci.* **24**, 933–937. https://doi.org/10.2135/cropsci1984.0011183X002400050026x (1984).
28. Fernandez, G. C. J. Effective selection criteria for assessing plant stress tolerance. In Adaptation of Food Crops to Temperature and Water Stress (eds. Kuo, C. G.) 257–270 (AVRDC, 1992).
29. Rosielle, A. A. & Hamblin, J. Theoretical aspects of selection for yield in stress and non-stress environments. *Crop Sci.* **21**, 943–946. https://doi.org/10.2135/cropsci1981.0011183X002100060033x (1981).

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Declarations

Competing interests

The authors declare no competing interests.

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