

Field Technique and Traits to Assess Reproductive Stage Cold Tolerance in Sorghum (*Sorghum bicolor* (L.) Moench)

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Abstract: Post-rainy season sorghum (*Sorghum bicolor* (L.) Moench) yields are often constrained by cold stress. Cold tolerance is a prerequisite for adaptation to post-rainy season. A study was conducted to develop a field screening technique for cold tolerance, and to identify traits that discriminate genotypes to cold response. A set of rainy and post-rainy season adapted sorghum genotypes were sown in 2010 – 2011 and 2011 – 2012 in 3 dates of sowing in post-rainy seasons from third week of October to second week of November. The average minimum temperature during a 30-day period after the initial 40-day vegetative growth was lower in October sowing, moderate in November sowing and high in December sowing. The panicle harvest index (PHI) was found to be sensitive to low temperatures and was identified as a stable trait for genetic discrimination. Reduction in PHI can be indicative of failure to set seed or poor grain filling. The genotypes bred for rainy season ICSB 52, ICSR 149 and ICSR 93034 exhibited reduced PHI and lighter seed in cold-prone environments, indicating that their sensitivity is also well reflected in the yields of ICSB 52 and ICSR 149. However, the genotypes bred for post-rainy season, Dagadi solapur, SPV 1411 and M 35-1, were not affected by cold, as indicted by their higher PHI and relatively less reduction in seed weight. It was suggested that PHI and seed weight together can be used as proxies in selecting for reproductive stage cold tolerance in sorghum.

Key words: Grain yield, Panicle harvest index, Photoperiod sensitivity, Post-rainy season-adaptation.

Both grain and stover of sorghum (*Sorghum bicolor* (L.) Moench) are valuable and used as food, feed, fuel and fiber (Clark et al., 1981; Paterson et al., 2009) with more than 35% of the world sorghum production being consumed in low-income food-deficit countries (Awika and Rooney, 2004). Generally, sorghum is a warm-season crop mainly grown in semiarid, sub-humid, and humid tropical and subtropical parts of the world where average minimum temperatures generally stay above 18°C during the growing season (Singh, 1985). However, a substantial amount of sorghum has been grown for centuries in the cooler highland areas of Burundi, Ethiopia, Rwanda, Uganda, and the Yemen Arab Republic in Africa. These sorghums are adapted to cold growing periods that should have merit in other parts of the world, especially the highlands of Latin America (Bolivia, Colombia, Ecuador, El Salvador, Guatemala, Honduras, Mexico and Peru), northern and northwestern USA, and southern Canada where rainfall is scarce and erratic, and where soils and growing conditions are generally less suitable for other major food and feed crops (Singh, 1985; Yu et al., 2004). In South Asia, particularly in India, major high quality

sorghum production comes from post-rainy season crop grown under receding soil moisture after the cessation of the rains with their peak flowering coinciding with the cooler days in winter. In this environment, reproductive success and ultimately grain yield is limited by poor seed setting (Reddy et al., 2012). However, early or late planting is not a viable option as its short day requirements and thermo-sensitivity affect either the shoot biomass productivity or partitioning (Tarumoto et al., 2005). Thus, low temperature tolerance during the early reproductive season, response to photoperiod sensitivity (short day length), flowering and maturity irrespective of temperature fluctuations and sowing dates (thermo-insensitivity), are critical for the post-rainy season crop (Craufurd et al., 1999; Reddy et al., 2010). Photoperiod sensitivity is a key feature that helps to adjust flowering to occur at a particular period irrespective of planting time and plays an important role in escaping from terminal drought, which is common in the post-rainy season conditions in India. It also helps to ensure good grain quality (Vaksmann et al., 1996), as the grain develops and matures in cold and dry conditions.

Table 1. Characteristics of sorghum genotypes evaluated for cold tolerance in 2010 – 2011 and 2011 – 2012 post-rainy seasons at ICRISAT, Patancheru.

Serial No	Genotypes	Main characteristics	Adapted season	Source
1	Dagadi solapur	Bold lustrous grain, high yielding	Post-rainy	MPKV, India
2	ICSB 52	High yielding, B-line	Rainy	ICRISAT, India
3	ICSR 149	High yielding, R-line	Rainy	ICRISAT, India
4	ICSR 93034	High yielding, R-line	Rainy	ICRISAT, India
5	M 35-1	Bold lustrous grain, high yielding	Post-rainy	Mohol, India
6	Parbhani jyothi	Bold grain, high yielding	Post-rainy	MAU, India
7	Phule anuradha	High yielding	Post-rainy	MPKV, India
8	Phule vasudha	High yielding	Post-rainy	MPKV, India
9	SPV 1359	High yielding	Post-rainy	MPKV, India
10	SPV 1411	Bold grain, high yielding	Post-rainy	MAU, India

Most available screening techniques and studies on cold tolerance are not suitable for adoption across the growing environments and most of them deal with seedling response to cold (Yu et al., 2004). Most of the genotypes that are tolerant at the vegetative stage do not show tolerance to cold during the reproductive stage (Singh, 1985). Therefore, developing a field screening technique to assess reproductive stage cold tolerance and to identify sources of tolerance is very crucial for enhancing the yield potential and stability, particularly in post-rainy season. The extent of success in ovule fertilization or ovule abortion is expected to be reflected by the panicle harvest index (PHI) in both lowland and upland rice (Lafitte et al., 2006). The 3 yield components i.e., weight of the panicle, PHI (ratio) and the filling of individual grains (seed weight) can help in indicating the reproductive success at the single plant level. The reduction of days to flowering in cold-prone environments helps in the adaptation to terminal stress. The objective of this work was to understand the field level cold tolerance, establish a simple and repeatable method for evaluating breeding lines and germplasm for reproductive stage cold tolerance and to identify sources with a high level of cold tolerance in the cultivated gene pool.

Materials and Methods

1. Experimental design

The experiments were conducted in the post-rainy seasons in 2010 – 2011 and 2011 – 2012, with 7 released varieties and 3 parental lines of sorghum (Table 1), in an Alfisol precision field at ICRISAT, Patancheru (17°30'N; 78°16'E; altitude 549 m) in peninsular India using a randomized complete block design (RCBD) with 6 replications. The genotypes used were known to flower between 65 to 80 days after sowing. Judging from the long-term averages of minimum temperature across the growing season, we planned to expose the crop sown early to cold and compare it with the performance of the crop sown later. The dates of sowing (DOS) were 23 October

(first DOS), 3 November (second DOS) and 13 November (third DOS) both in 2010 – 2011 and 2011 – 2012 seasons, with the coolest (minimum temperature) peak flowering time in the first DOS, and relatively warmer flowering period in the third DOS; and second DOS was used in case there was a delay in arrival of low temperatures. The meteorological data for the entire crop period from 23 October (43rd standard week) to 31 March (13th standard week) of the succeeding year in both periods were obtained from the ICRISAT, Patancheru weather station situated 600 m away from the experimental field. The soil depths of the fields varied from 0.8 to 1.0 m and were prepared into 0.75 m ridges and furrows. The plots were 2 rows 2 m long with 0.15 m intra-row spacing between plants in both seasons. Seeds were treated with 70% (water soluble) of Imidacloprid (Sensex® GSP Crop Science Pvt Ltd) in the 2 seasons to protect the crop from sorghum shoot fly. Surface application and incorporation of 40 kg N ha⁻¹ and 40 kg P ha⁻¹ fertilizers as basal, and 40 kg N ha⁻¹ at 30 days as top dressing, was carried out. The trials were optimally irrigated receiving irrigations on 1, 18, 33, 52, 68, 82, 93 and 105 days after sowing in the first DOS, 1, 19, 35, 52, 68, 82, 92 and 105 days after sowing in the second DOS and 1, 22, 36, 51, 70, 82, 91 and 102 days after sowing in the third DOS.

We recorded the traits, plant height, days to 50% flowering, days to maturity and grain yield in the whole plot after removing one border plant on either side of the rows. At the time of anthesis, 10 panicles that represent the time of flowering and panicle weight of the whole plot were tagged and the panicles were harvested separately and used for recording panicle weight before threshing and grain weight after threshing. Panicle morphology reflects the stresses encountered during the formation and development and how that particular genotype responded to the stress. PHI is calculated as follows:

$$\text{PHI} = \text{grain dry weight} / \text{panicle dry weight}$$

All the grains from the individual panicles were counted for estimating the seed weight.

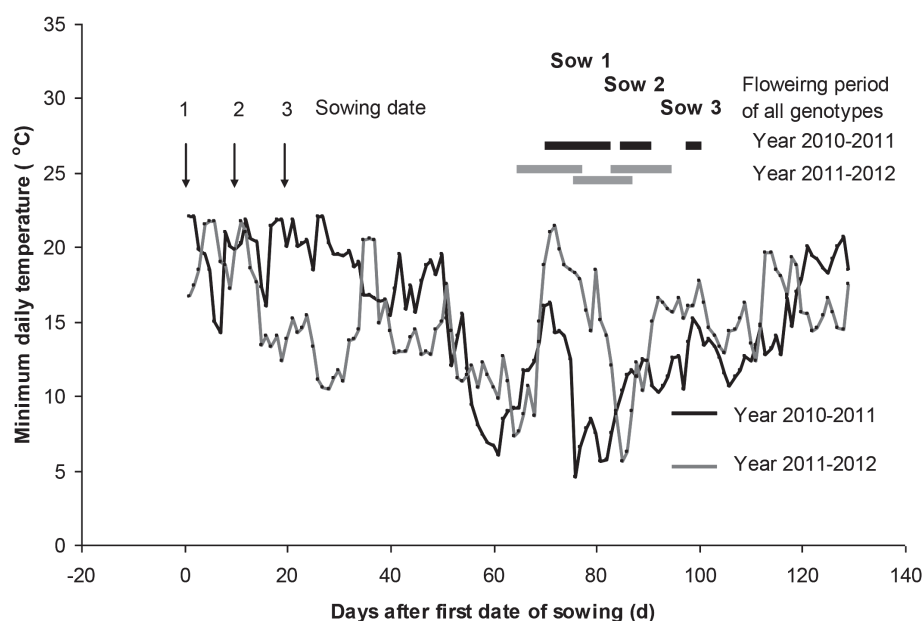


Fig. 1. Minimum temperature prevailed during the post-rainy cropping season of 2010 – 2011 and 2011 – 2012.

Table 2. Mean minimum temperature (°C) prevalent 3 days before and 3 days after 50% anthesis of individual genotypes grown in 3 dates of sowing (DOS) at ICRISAT, Patancheru during 2010 – 2011 and 2011 – 2012 post-rainy seasons.

Genotypes	2010 – 2011			2011 – 2012		
	DOS I	DOS II	DOS III	DOS I	DOS II	DOS III
Dagadi solapur	8.7	11.3	13.5	19.5	14.0	10.1
ICSB 52	13.1	8.7	13.8	9.9	14.0	15.8
ICSR 149	11.7	10.5	13.8	9.9	15.3	16.0
ICSR 93034	9.7	11.4	13.4	16.5	11.4	15.8
M 35-1	8.7	10.5	13.5	17.6	12.6	15.4
Parbhani jyothi	9.6	11.3	13.5	16.9	11.4	10.1
Phule anuradha	14.4	11.3	13.4	14.9	9.2	15.8
Phule vasudha	6.6	11.3	13.5	19.0	14.0	14.0
SPV 1359	7.0	11.3	13.8	18.6	9.7	15.9
SPV 1411	7.7	11.3	13.5	17.4	10.1	16.0

2. Statistical analysis

The computed mean values of data recorded on sample plants in 6 replications for agronomic traits were used for statistical analysis. All the statistical analyses were carried out using GENSTAT 14th version of Rothamsted Research Station. Analysis of variance as an RCBD with 2 factors, was used to assess the effect of genotype, DOS, year and genotype \times DOS \times year interactions. Multiple linear regression analysis was carried out independently on different DOS considering yield as the dependent variable and the yield components, PHI, panicle weight and seed weight as the independent variables.

Results

Minimum temperatures and other climatic factors in relation to sowing are shown in Fig. 1. In the 2010 – 2011

season, the first DOS crop experienced lowest minimum temperature during panicle development and flowering compared to other dates. However, it was the second sown crop that experienced lowest minimum temperature during 2011 – 2012 while 2 early genotypes ICSB 52 and ICSR 149 experienced low minimum temperatures at anthesis (Table 2). Although the characteristic minimum temperature dip (peak winter) occurred from the third week of December to second week of January, there was a warm weather peak for one week in 2010 – 2011 and for 2 weeks in 2011 – 2012 (Fig. 1). Therefore, in the first DOS, most genotypes flowered and experienced cooler temperatures in some part of their early panicle formation and development. Panicle weight, PHI and seed weight were considered as 3 independent components of grain yield and days to 50 % flowering, plant height were other

Table 3. Analysis of variance for the traits measured in sorghum, 2010 – 2011 and 2011 – 2012 post-rainy seasons at ICRISAT, Patancheru.

Source of variation	d.f.	Panicle weight (g)	Panicle harvest index	Seed weight (g 100 ⁻¹)	Days to 50% flowering	Grain yield (t ha ⁻¹)	Plant height (cm)
Year	1	1346**	0.0006 ^{Ns}	0.90**	4898.8**	1.71 ^{Ns}	61413**
DOS	2	5809**	0.0782**	2.84**	23.3**	18.56**	30002**
Genotype	9	4317**	0.0503**	4.06**	149.1**	6.76**	68007**
Year × DOS	2	2634**	0.0052**	2.69**	116.6**	7.07**	4876**
DOS × Genotype	18	757**	0.0196**	0.69**	80.6**	2.24**	2613**
Year × Genotype	9	510**	0.0023**	0.19	31.9**	0.48 ^{Ns}	8698**
Year × DOS × Genotype	18	534**	0.0023**	0.36**	28.3**	0.61 ^{Ns}	1658**

** = Significant at $p = 0.01$, ^{Ns} = Nonsignificant.

yield-influencing characters that were studied. By the analysis of variance (ANOVA), effects of year, DOS, genotypes, year × DOS, DOS × genotypes, year × genotypes and the year × DOS × genotypes were significantly varied except the year effect for the PHI, year × genotypes effect for seed weight and year, year × genotypes, year × DOS × genotypes effects for the grain yield (Table 3).

1. Days to 50% flowering

Mean flowering time was advanced in 2011 – 2012 to 70 days compared to 77 days in 2010 – 2011. The flowering time of genotypes ranged from 70 to 84 days in 2010 – 2011 and 65 to 77 days in 2011 – 2012 in the first DOS. Most genotypes delayed their flowering in the first sowing of first year due to relatively warmer (minimum) temperature that prevailed during vegetative stages (Fig. 1). The genotypes formed in to three distinct flowering groups (Fig. 2). Interestingly, the genotypes bred for the rainy season and post-rainy season adaptation were found to respond differently to different DOS. The rainy season genotypes ICSB 52, ICSR 93034 and ICSR 149 flowered early in the first DOS but with relative delays with the delay in DOS. As the temperature changes appeared faster than the photoperiod at this time of the year, these genotypes are likely to be thermo-sensitive. However, post-rainy sorghums such as Dagadi solapur, Parbhani jyothei and SPV 1411 flowered late when sown normal but advanced their flowering with further delay in sowing and are likely to be temperature-insensitive. This mechanism helps them to escape from terminal drought in the post-rainy season especially when the DOS is late in India. On the other hand, the delayed maturity cycle in the first DOS helps them to yield more by taking advantage for the moisture available in the deeper layers of the soil. However, the genotypes like M 35-1, Phule vasudha and SPV 1359 were the most stable genotypes in all DOS forming the third group that are likely to be insensitive to photoperiod and temperature.

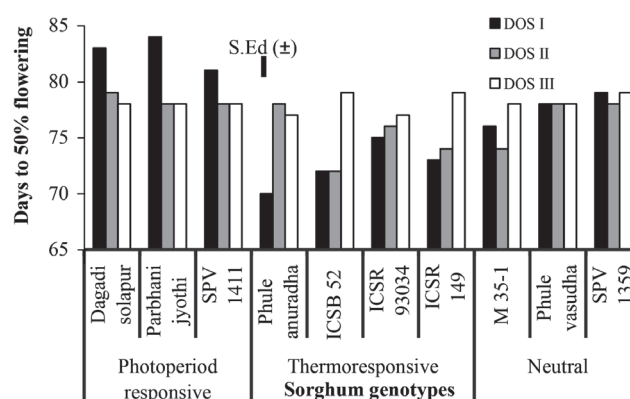


Fig. 2. Sorghum genotypic response to 50% flowering under 3 different date of sowings (DOS) in 2010 – 2011 post-rainy season at ICRISAT, Patancheru. The DOS, genotypes and DOS × genotype interactions were significant at $p = 0.01$ level. Vertical bar denotes the standard error of difference (1.7 day) for comparison of DOS × genotype means.

2. Plant height

Mean plant height of genotypes ranged from 153 to 280 cm in 2010 – 2011 and 150 to 317 cm in 2011 – 2012 in the first DOS. There was a considerable reduction of plant height with the delay in DOS in both the years. Genotype SPV 1359 was the tallest in first season. However genotype Parbhani jyothei was tallest in the second season. In general the rainy season-adapted ICSB 52, ICSR 149 and ICSR 93034 were shorter in height in all DOS while the post-rainy season-adapted genotypes Dagadi solapur, Parbhani jyothei, Phule vasudha, SPV 1411 and SPV 1359 were relatively taller, about 2.5 m. Delay in sowing in general reduced the plant height (Data not shown). For higher yield, genotypes with a plant height of around 1.5 m seem to be desirable.

3. Grain yield

There was no significant difference in yield between seasons in 2010 – 2011 and 2011 – 2012 or among the

Table 4. Variation in grain yield (t ha^{-1}) of 10 sorghum genotypes grown in 3 dates of sowing (DOS) at ICRISAT, Patancheru during 2010 – 2011 and 2011 – 2012 post-rainy seasons.

Genotypes	2010 – 2011				2011 – 2012			
	DOS I	DOS II	DOS III	Mean	DOS I	DOS II	DOS III	Mean
Dagadi solapur	4.90	3.53	2.77	3.73	4.65	2.92	4.15	3.91
ICSB 52	2.26	1.86	2.90	2.34	2.10	1.99	2.85	2.31
ICSR 149	3.20	1.99	3.38	2.86	3.04	2.02	3.21	2.76
ICSR 93034	3.60	2.03	3.07	2.90	3.28	2.56	3.55	3.13
M 35-1	3.21	3.33	2.22	2.92	2.90	2.48	3.42	2.93
Parbhani jyothis	3.76	2.65	2.81	3.07	3.44	2.32	3.56	3.11
Phule anuradha	3.07	3.16	2.32	2.85	3.69	3.70	3.28	3.56
Phule vasudha	3.59	3.56	2.62	3.26	3.68	3.24	3.43	3.45
SPV 1359	4.38	3.62	2.69	3.56	4.35	3.15	3.79	3.76
SPV 1411	4.30	3.02	2.96	3.43	4.05	2.64	3.47	3.39
Mean	3.63	2.88	2.77	–	3.52	2.70	3.47	–
Standard error of difference for comparison of means of								
DOS \times Genotype	0.334							
Genotype \times Year \times DOS	0.473							
Coefficient of variation (%)	13.0							

genotypes, but there was a significant difference between different DOS in each season. This clearly indicates the prevalence of low temperature and its magnitude towards reproductive success in terms of grain yield. Mean grain yield ranged from 2.3 to 4.9 t ha^{-1} in 2010 – 2011 and 2.2 to 4.7 t ha^{-1} in 2011 – 2012, in the cold prone first DOS. In the first DOS, genotype Dagadi solapur yielded highest in both seasons, followed by genotypes SPV 1359, SPV 1411, Parbhani jyothis, Phule vasudha and Phule anuradha in this order (Table 4); these were all post-rainy season adapted cultivars. However, rainy season sorghums such as ICSB 52 in the first year and ICSB 52, ICSR 149 and ICSR 93034 in the second year were the poorest yielders among the first-sown. The rainy season genotypes tended to show a decrease in yield in the second DOS whereas in the third DOS it remained close to the first DOS yield or marginally increased. However, post-rainy season genotypes, in general, showed decreased yield when sown later. This indicated that rainy season-adapted plants do not possess cold tolerance and are temperature-sensitive thriving well under warmer conditions without changing their flowering time. In contrast, post-rainy season-adapted materials possess cold tolerance and give rise good yields but being photoperiod sensitive tend to flower early and cannot exploit the warmer temperature. However in a typical post-rainy season ecology terminal moisture stress is the major issue (Reddy et al., 2012). Thus, genotypes with cold tolerance and photoperiod sensitivity only can thrive well.

4. Panicle weight

The panicle weight was reduced with the delay in sowing

and the genotype Dagadi solapur produced the largest panicle in the first DOS with lighter panicle in the third DOS during both years (Table 5). Mean panicle weight of genotypes ranged from 62 to 137 g in 2010 – 2011 and 54 to 99 g in 2011 – 2012 in the first DOS. SPV 1411 had a combined advantage of minimum reduction in panicle weight with delayed sowing and larger panicle weight in all DOS. The panicles of ICSB 52, ICSR 93034 and ICSR 149 were smaller compared to others during both the years. Overall, the panicle weights of the genotypes were lighter in the second DOS. The panicle weight of Phule anuradha and SPV 1359 were lighter in the third DOS in 2010 – 2011 while no such reduction was noticed in 2011 – 2012. The genotypes Dagadi solapur produced the largest panicle in cold prone environments in both seasons followed by Parbhani jyothis, Phule vasudha and SPV 1411.

5. PHI

The PHI, a measure of efficiency in seed set and seed filling, tended to be the highest in the second or third DOS (Table 6). There were no significant changes in PHI over the years; PHI remained the same across years, increased with the delay in DOS with the genotypes behaving differently across DOS. Mean PHI of genotypes ranged from 0.63 to 0.85 in 2010 – 2011 and 0.63 to 0.84 in 2011 – 2012 in the cold prone first DOS. The genotypes like ICSB 52, ICSR 93034 and ICSR 149 had poor PHI in the first sowing indicating that these are cold sensitive. Phule anuradha, Parbhani jyothis, Phule vasudha and SPV 1411 showed consistent PHI across all the sowing times. Genotypes such as Dagadi solapur and M 35-1 in 2010 –

Table 5. Variation in panicle weight (g) of 10 sorghum genotypes grown in 3 dates of sowing (DOS) at ICRISAT, Patancheru during 2010 – 2011 and 2011 – 2012 post-rainy seasons.

Genotypes	2010 – 2011				2011 – 2012			
	DOS I	DOS II	DOS III	Mean	DOS I	DOS II	DOS III	Mean
Dagadi solapur	137.2	103.1	45.0	95.1	98.7	88.7	83.4	90.3
ICSB 52	62.8	53.7	61.6	59.4	61.9	58.8	56.1	59.0
ICSR 149	62.4	56.1	56.2	58.2	62.1	57.8	56.8	58.9
ICSR 93034	69.6	48.3	56.7	58.2	68.2	48.0	52.6	56.3
M 35-1	77.9	69.0	59.1	68.7	53.7	49.8	51.5	51.7
Parbhani jyothi	81.2	74.0	61.2	72.1	60.3	56.5	60.3	59.0
Phule anuradha	69.7	60.5	58.9	63.0	77.7	71.7	69.1	72.9
Phule vasudha	76.8	77.9	62.8	72.5	74.0	72.5	72.0	72.8
SPV 1359	83.0	87.8	54.8	75.2	67.5	66.8	74.0	69.4
SPV 1411	88.2	86.7	65.7	80.2	76.8	70.2	74.0	73.7
Mean	80.9	71.1	58.2	–	70.1	64.1	65.0	–
Standard error of difference for comparison of means of								
DOS × Genotype			4.92					
Genotype × Year × DOS			6.95					
Coefficient of variation (%)			15.1					

Table 6. Variation in panicle harvest index of 10 sorghum genotypes grown in 3 dates of sowing (DOS) at ICRISAT, Patancheru during 2010 – 2011 and 2011 – 2012 post-rainy seasons.

Genotypes	2010 – 2011				2011 – 2012			
	DOS I	DOS II	DOS III	Mean	DOS I	DOS II	DOS III	Mean
Dagadi solapur	0.850	0.846	0.771	0.822	0.837	0.823	0.804	0.821
ICSB 52	0.628	0.717	0.800	0.715	0.626	0.699	0.780	0.702
ICSR 149	0.631	0.779	0.788	0.733	0.634	0.773	0.797	0.735
ICSR 93034	0.690	0.764	0.792	0.749	0.672	0.761	0.787	0.740
M 35-1	0.787	0.833	0.727	0.782	0.804	0.803	0.797	0.801
Parbhani jyothi	0.766	0.745	0.801	0.771	0.781	0.804	0.779	0.788
Phule anuradha	0.783	0.791	0.778	0.784	0.746	0.763	0.790	0.766
Phule vasudha	0.739	0.818	0.792	0.783	0.744	0.809	0.796	0.783
SPV 1359	0.775	0.839	0.770	0.795	0.796	0.780	0.792	0.789
SPV 1411	0.797	0.838	0.793	0.809	0.837	0.848	0.845	0.843
Mean	0.745	0.797	0.781	–	0.748	0.786	0.797	–
Standard error of difference for comparison of means of								
DOS × Genotype			0.0237					
Genotype × Year × DOS			0.0335					
Coefficient of variation (%)			2.6					

2011 were not seemingly affected by cold whereas their PHI seem to reduce with warmer temperature that were experienced with the third DOS. The flowering in some sorghum genotypes are likely to be controlled by interactive operation between the photoperiod sensitive genes and thermo sensitive genes. This might be one of the reasons that those genotypes showed PHI reduction in the third sowing (Tarumoto et al., 2005).

6. Seed weight

Mean seed weight of genotypes remained constant over the seasons. However, seed weight of rainy season adapted materials decreased in the first DOS compared to the third DOS. However, in the post-rainy season-adapted materials, seed weight decreased in delayed sowing dates only in 2010 – 2011 (Table 7). Post-rainy season-adapted genotypes M 35-1 and SPV 1359 also showed decrease in seed weight in

Table 7. Variation in 100 seed weight (g) of 10 sorghum genotypes grown in 3 dates of sowing (DOS) at ICRISAT, Patancheru during 2010 – 2011 and 2011 – 2012 post-rainy seasons.

Genotypes	2010 – 2011				2011 – 2012			
	DOS I	DOS II	DOS III	Mean	DOS I	DOS II	DOS III	Mean
Dagadi solapur	3.76	3.48	3.13	3.45	2.91	3.20	3.49	3.20
ICSB 52	3.08	3.88	3.78	3.58	3.15	3.65	3.72	3.51
ICSR 149	2.44	2.55	3.17	2.72	2.53	2.63	3.13	2.76
ICSR 93034	2.18	2.58	3.45	2.74	2.29	2.75	3.21	2.75
M 35-1	3.61	3.41	3.47	3.50	2.85	3.52	3.34	3.23
Parbhani jyothei	4.01	3.03	3.71	3.58	3.32	3.81	3.65	3.60
Phule anuradha	3.60	3.79	3.46	3.62	3.20	4.22	3.39	3.61
Phule vasudha	3.65	3.36	3.44	3.48	3.28	3.68	3.57	3.51
SPV 1359	3.69	3.64	3.38	3.57	3.16	3.56	3.56	3.43
SPV 1411	3.98	3.68	3.72	3.79	2.91	3.70	3.70	3.44
Mean	3.40	3.34	3.47	–	2.96	3.47	3.48	–
Standard error of difference for comparison of means of								
DOS × Genotype			0.145					
Genotype × Year × DOS			0.204					
Coefficient of variation (%)			9.4					

the first DOS. Genotype Phule anuradha produced very high seed weight in the second DOS that is difficult to explain. There was a reduction of seed weight between the two seasons for first DOS indicating prevalence of relatively low temperature in the second season i.e., 2011 – 2012. Mean seed weight of genotypes ranged from 2.2 to 4.0 g 100⁻¹ in 2010 – 2011 and 2.3 to 3.3 g 100⁻¹ in 2011 – 2012 in the cold prone first DOS. The genotypes Dagadi solapur in 2010 – 2011 and Parbhani jyothei, Phule anuradha, Phule vasudha, SPV 1359 and SPV 1411 in both seasons recorded higher seed weight in the first DOS. In both seasons, experiments revealed the moderate increase in seed weight during late sowing. The seed weight of Phule anuradha, M 35-1 and SPV 1359 were not affected by DOS whereas that of ICSB 52, ICSR 93034 and ICSR 149 was adversely affected confirming their cold sensitivity. Interestingly the genotype Dagadi solapur showed the highest seed weight in the first DOS.

7. Association of yield components with grain yield

In the first DOS, both panicle weight and PHI were closely associated with the grain yield consistently in both seasons (Fig. 3). Though the seed weight was not associated with the grain yield, the varieties that produced larger seeds (Dagadi solapur, SPV 1359 and SPV 1411) also produced the highest grain yields (Fig. 3). However, such association of panicle weight and PHI turned inconsistent in the second DOS. In the second DOS, the panicle weight association was significant in 2010 – 2011 ($r^2 = 0.62$, $p \leq 0.0$) but not in 2011 – 2012 ($r^2 = 0.32$, $p = ns$) (data not shown). Similarly for PHI r^2 was 0.70 ($p \leq 0.01$) in 2010 – 2011 but

was 0.07 ($p = ns$) in 2011-12. This association turned inconsistent and clearly non-associated for both panicle weight (2010 – 2011 $r^2 = 0.00$, $p = ns$; 2011 – 2012 $r^2 = 0.39$, $p = ns$) and PHI (2010 – 2011 $r^2 = 0.41$, $p = ns$; 2011 – 2012 $r^2 = 0.05$, $p = ns$) in the third DOS (data not shown).

Discussion

Sorghum adapts well to warmer temperatures than most other cereal grain crops, but exhibits less tolerance to cold (Rooney, 2004). In the agricultural ecosystems such as Mediterranean-type climates and the South Asian post-rainy season (winter) where the crops are grown under terminal drought and rely on stored soil moisture, higher grain yields in sorghum are possible only when the flowering time of any proposed variety coincides with the peak cold for achieving a balanced vegetative and reproductive periods of growth. Also the crop experiences short-days and low temperature in December – January when the flowering occurs. Post-rainy season sorghum needs to be planted in the last week of October and the exposure of flowering phase to cold can not be avoided. This is because an earlier sowing will expose the crop to the attack by shoot flies and extend the flowering time of photoperiod-sensitive sorghum leading to flowering once again at cold and a later planting will reduce plant size and expose plants to higher intensities of drought stress with the onset of summer (Reddy et al., 2012). Therefore fitting the phenology of sorghum to the available soil moisture window of the season requires choice of sorghum with about equal duration before and after the flowering as well as exposing the flowering to peak cold of the region

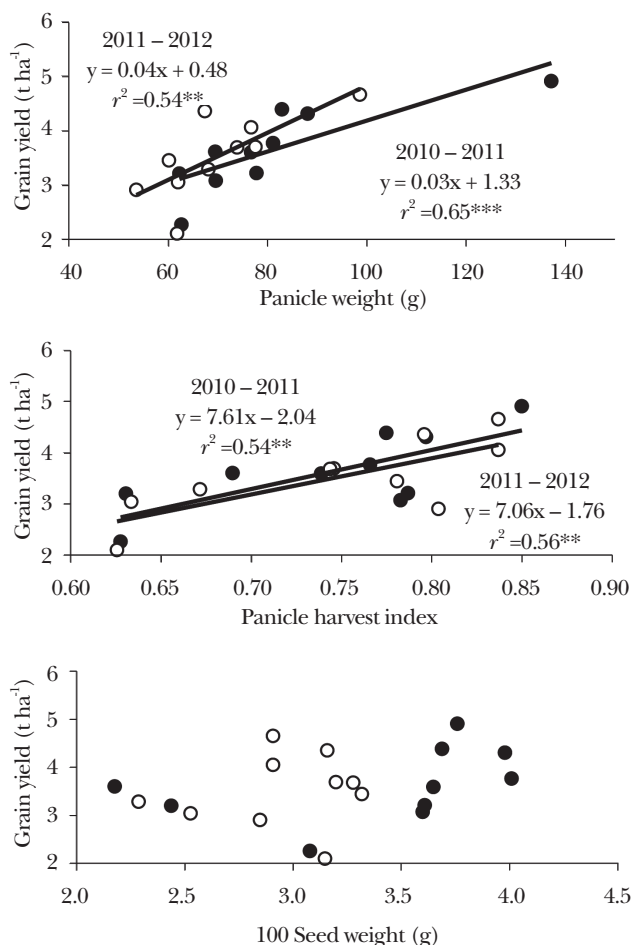


Fig. 3. Relationship of panicle weight, panicle harvest index and the seed weight with the grain yield in DOS I of both 2010 – 2011 and 2011 – 2012 post-rainy seasons. The r^2 values marked with ** denotes significance at $p = 0.01$, *** denotes significance at $p = 0.001$ level and no marking denotes no significance.

(Reddy et al., 2012). The genotypic cold tolerance during the vegetative stage is independent of cold tolerance during the reproductive stage and cold tolerance appeared to be a dominant trait. However, several genes control the inheritance of the character and their effects are largely cumulative (Singh, 1985). Therefore, it is important to develop a highly reproducible protocol for assessing reproductive stage cold tolerance, within the same field trials that are intended for grain yield selection. This study showed that measurement of PHI and 100 seed weight would give a good indication of reproductive stage cold tolerance in sorghum.

1. Flowering

Cold causes large changes in vegetative versus reproductive growth patterns. Low temperature stress delays panicle emergence and heading in sorghum (Majora et al., 1982). Even crops inherently tolerant to low temperature do suffer from cold stress during flowering (Maqbool et al., 2010). As a consequence, sorghum

cultivars produced more leaves (Hesketh et al., 1969; Quinby et al., 1973), delayed floral initiation (Caddel and Weibel, 1971; Quinby et al., 1973), and found to induce male sterility (Downes and Marshall, 1971; Singh, 1977) when grown under low temperatures. In such screening efforts where the plants of a critical stage of crop growth is expected to be subjected to cold, further precision can be achieved by synchronizing the stage of the plants to a narrow window of calendar days, by staggered sowing. The test genotypes can be grouped on their days to 50% flowering and sown at different DOS, so that the flowering time of the genotypes synchronize within a small window of calendar days and experience prevailing low temperature (Singh, 1985) and at the same time, helping to understand the basis of photoperiod sensitivity and thermo-sensitivity (Tarumoto et al. 2005). In West Africa and also in post-rainy season in India this mechanism works particularly well as the end of the season is far more predictable (and less variable) than the start (Craufurd et al., 2011). The present study confirms the recognition by Reddy et al. (1987) of M35-1, the post-rainy season ruling variety, as a photoperiod- and thermo-insensitive variety having a distinct ability to finish flowering in more or less the same time. M 35-1 showed the same time to flowering even in delayed sowing (Fig. 2). Further breeding efforts, following this recognition involving M 35-1 as a parent, have led to the development of several successful improved progenies for post-rainy season adaptation (Reddy et al., 2009).

2. Yield components

Exposure of plants to cold stress during the reproductive stage has tremendous effects on yield components (Nishiyama, 1995). The yield reduction due to low temperature in sorghum was associated with an increase in pollen sterility during panicle development by 20%, with an increase in floret abortion with shriveled anthers and withering stigmas by 23% and a reduction in the number of seeds by 39% (Dhopte and Eastin, 1990, 1992). Low temperature stress at the reproductive stage causes flower abscission, pollen sterility, pollen tube distortion, ovule abortion and poor seed-set (Thakur et al., 2010). Subjecting plants to cold stress significantly reduces spikelet fertility in rice (Pereira da Cruz et al., 2006). However, in sorghum, the floral initials form well before the panicle emergence, i.e. 30 to 40 days after germination (House, 1985), and the number of florets, panicle weight will be decided much earlier and are prone for response to cold. Therefore the panicle weight, as seen in the third sowing in 2010 – 2011 (Table 5), can be adversely affected. However, the seed set after fertilization and the seed filling starts after flowering. This period is very sensitive to cold (Dhopte and Eastin, 1990, 1992) and this reproductive success determines the PHI and the seed weight. After fertilization, the partitioning of the photosynthates from

source to sink is determined by genotypic response to cold stress. Such genetic variation in photosynthate partitioning seems to be available in the form of PHI and seed weight for exploitation as traits. Furthermore, grain yield in sorghum under low temperature conditions was shown to be positively correlated with harvest index (HI) and 1000 grain mass (Patil et al., 2004).

The genotypes Dagadi solapur, SPV 1411 and M 35-1 were not affected by cold, as indicated by their higher mean panicle weight (Table 5) and higher PHI (Table 6) in the cold prone environments. The genotype Dagadi solapur yielded highest under the cold-prone environment followed by SPV 1359, SPV 1411, Parbhani jyothe, Phule vasudha and Phule anuradha (Table 4). However, genotypes ICSB 52, ICSR 93034 and ICSR 149 were sensitive to cold with variable flowering periods (Fig. 2) and lighter panicle weight and lower PHI in the cold environments (Table 5, 6) indicating their cold sensitivity. The grain yield data of the first DOS across years, the usual planting time in this region, confirm the above results i.e., ICSB 52, ICSR 149 and ICSR 93034 the rainy season bred sorghums, are poor yielders in the post-rainy season (Table 4) as they are sensitive to temperature.

3. PHI

PHI is a trait found to substantially explain terminal drought tolerance in pearl millet among other crops - a dryland crop similar to sorghum. Various yield components along with grain yield measured under different watering regimes, using some varieties differing in their tolerance to terminal drought and subsequent analysis, revealed that the number of grains per panicle and the 100-grain weight were the yield components most affected under terminal drought conditions, leading to a decrease in PHI (Bidingier et al., 1987). The PHI is also the threshing index, and represents the proportion of grain weight per whole panicle. A high PHI means that most florets of the panicle have successfully produced a grain, and that this grain has filled up to its potential. Since cold is known to adversely affect reproductive success, we measured PHI and found it to be sensitive to low temperatures and show a good level of genetic discrimination. A maximum PHI of 0.85 is attainable under stress-free environments. Reduction of this value can be indicative of either failure in seed set in some spikelets, or alternatively, failure of the grains to fill to their potential. In earlier studies PHI was found to be closely correlated with spikelet fertility in sorghum (Dhopte and Eastin, 1990, 1992), in rice (Lafitte et al., 2006; Pereira da Cruz et al., 2006) and in wheat (Subedi et al., 1998). PHI did not change across years in this study indicating its stability. This was obvious from the mean sum of squares distribution for PHI (Table 3). The major portion of the mean sum of squares was shared by the DOS and the genotypes with very small 2 or 3 treatment factor

interaction mean sum of squares. Furthermore, multiple linear regression estimates have shown that PHI did contribute to the yield only in the cold prone first DOS (Fig. 3). Both reduced PHI and seed weight, compared to the third DOS, have been seen in genotypes of ICSB 52, ICSR 149 and ICSR 93034 that reflected very well on the yields of ICSB 52 and ICSR 149. Also it is apparent that the reproductive success or failure is one major adaptation variation among the genotypes to seasons. Therefore recording of one more variable in yield evaluation trials, i.e. the PHI, should ensure a simultaneous selection for yield as well as cold tolerance. However, further work involving larger number of germplasm is needed to confirm the robustness of this screening protocol.

The majority of the cold-tolerant sorghum genotypes from this study are released varieties from Mahatma Phule Krishi Vidyapeeth, Rahuri (Dagadi solapur, Phule anuradha, Phule vasudha and SPV 1359) and Marathwada Agricultural University, Parbhani (Parbhani jyothe and SPV 1411). M 35-1 is a selection made from maldandi, a local accession, in 1937 at Mohol region of Maharashtra. It is a variety popular with farmers for its grain quality and adaptation in post-rainy season. Maharashtra is the major sorghum growing state of India and the agro-ecology is ideally suited for sorghum cultivation. The days are warm and bright but the minimum temperatures are known to go below 10°C for at least 7 weeks and below 5°C for at least a week in winter indicating that winter is moderately cold (Jahagirdar and Borikar, 2002). Most varieties bred and selected in these regions seem to have the best cold tolerance and this adaptation also seems to be responsible for the best performance of Dagadi solapur. It showed the highest yield in the cold-prone first DOS or the normal post-rainy season sowing and this variety seems to be successful in developing a large panicle during a cold period. This region could serve as a hot spot for screening and breeding for cold tolerance and stored soil moisture adaptations.

Conclusion

The seed set and grain filling under low temperature conditions, as shown by PHI and 100 seed weight, indicates the level of cold tolerance in sorghum and provides good levels of genotypic discrimination. The genotype, Dagadi solapur is ideal for use as a cold-tolerant control variety in multi-location yield trials under low temperature conditions. This variety can also help breed improved genotypes of sorghum with higher PHI, increased cold tolerance and the best post-rainy season adaptation. This simple and cost-effective screening protocol will make it possible to evaluate yield and cold tolerance simultaneously in field trials.

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