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Response of diverse groups of sorghum [*Sorghum bicolor* (L.) Moench] genotypes to low temperature stress at anthesis

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Abstract

Rapid productivity increases has not been realized in winter/ post-rainy/*rabi* sorghum, an important food crop in the semi-arid tropical regions of India. The crop is dominated with varieties as poor seed set due to susceptibility to cold stress is noticed in the hybrids. Hence, before initiation of breeding program to bring in tolerance to cold stress, diverse group of breeding material that included 8 varieties, 7 maintainer lines, 5 restorer lines and a hybrid were screened for tolerance to cold stress in two dates of sowing for pollen viability and germinability apart from other agronomic traits. Date of sowing significantly influenced pollen germinability and the influence of the cooler night temperatures was more than the genetic background. In contrast, pollen viability had less phenotypic plasticity and highly heritable. The loss of grain yield (open panicle grain yield-selfed panicle grain yield) was about 5g/panicle in varieties while it was 1.9g/panicle in restorer lines and about 5.7g/panicle in B-lines. The loss in grain yield was highest in the hybrid at about 22.9g. Higher grain yields can be attained in the hybrid with improvement in the pollen viability and germinability. The germinability percentage was positively associated with selfed panicle weight, grain yield and harvest index while the viability percentage did not show such association. The germinability percentage was more linearly related in R lines followed by B lines and varieties.

Key words: Sorghum, cold stress, pollen viability, seed set

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is a staple food crop for millions of poor in the semi-arid tropics (SAT) of Asia with India having the largest area under sorghum [1]. In India, it is grown during both rainy/ *kharif* (June-October) and winter/post-rainy/*rabi* (October- January) seasons for multiple uses as a food, feed, fodder and a fuel crop. However, the *rabi* sorghum assumes importance from the perspective of food for poor people in semi-arid tropical regions of the country. The *rabi* sorghum is grown in receding soil moisture under relatively cooler climatic conditions. Though productivity recorded a high compound growth rate of 2.5% per annum in *kharif* sorghum, there has been wide productivity differential between *kharif* and *rabi* sorghums by 2 to 3 times [2]. Non availability of sorghum hybrids for *rabi* season and cultivation under residual and receding soil moisture are considered as major factors for non-realization of the productivity potential. One of the reasons for the poor yield performance of hybrids is poor seed setting mainly caused due to low temperatures prevailing during anthesis especially when the *kharif* and *rabi* sorghum derivatives are used in parental lines.

Owing to its origin in the tropical and sub-tropical regions of Africa, sorghum is well adapted to warm growing conditions. Since sorghum has tropical origin, it is considered as a cold-susceptible species except for the cold-tolerant germplasm. Ecological male-sterility is reported in sorghum wherein cold-sensitive genotypes have lower pollen production and viability when the temperatures decreases below 12°C [3, 4] or 16°C [5]. Interaction of viable pollen production or pollen shedding with temperature was reported by Stephens and Holland as early as 1954 itself. More specifically, when minimum temperature goes below 10°C for several days during flowering, the hybrids that are otherwise male fertile show male sterility [6]. Conversely, other studies on sorghum suggest that the ensuing reproductive stages from meiosis in spore

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mother cells to fertilization and seed development are more susceptible to cold stress [7]. Fertile pollen grains have a denser cytoplasm than that of sterile ones with the latter in the extreme case, appearing completely empty. This condition has been used as a criterion to score and quantify the degree of tolerance or susceptibility to low temperatures of sorghum genotypes [8]. Similarly, the percentage of grain set in selfed panicles has also been used as a criterion for this purpose [9].

Screening under controlled environments [10] with a low night temperatures of 13^{o}C [11] and field screenings using naturally occurring cold temperatures [4, 12] have been used by researchers to evaluate sorghum genotypes for cold stress. However, relatively little information is available on response to cold stress in diverse groups of sorghum genotypes such as maintainer lines, restorer lines, varieties and hybrids. An experiment was therefore conducted i) to study the effect of low temperature stress on pollen viability and germinability and seed set percentage in diverse groups of sorghum genotypes and ii) to determine the relationship of pollen traits (viability and germinability) with other agronomic traits.

Materials and methods

Genetic material

The material for the study included 21 genotypes of sorghum which included seven *rabi* season adapted varieties (M 35-1, DSV 4, CSV 216R, Phule Anuradha, Phule Maulee, Phule Chitra, Parbhani Moti), a rainy season adapted variety (C 43), seven maintainer lines on A_1 cytoplasm (B-lines) (104B, 1409B, 296B, M 31-2B, AKRB 306, AKMS 14B, B 35), five restorer lines (R-lines) (AKR 150, AKR 354, RS 585, SLR 5, SLR 46) and a *rabi* hybrid (CSH 15R).

Experimentation

The genotypes were planted in a randomized complete block design with two replications and in two dates of sowing (20 Oct 2011 and 7 Nov 2011). The sowing was by overseeding and the plants were thinned to one plant every 15 cm. Each plot consisted of two rows of 2m length with 45cm between rows. Of the two rows, one row was selfed and the other was left for open pollination. The data recording period in the first sowing date experienced an average minimum night temperature of 16^{o}C and second sowing date experienced an average minimum night temperature of 13^{o}C.

Data recording

The observations on pollen were recorded at 50% flowering while yield and yield components were recorded at harvest (ten days after physiological maturity). Pollen viability was estimated using acetocarmine [13]. To estimate pollen viability, pollen was collected in the morning hours from the plant which showed full blooming. The pollen was placed on a slide containing 2-3 drops of acetocarmine stain (2%) and left for 2-3 minutes for proper staining. The cover slip was then placed and examined under microscope for staining pattern. The pollen grains which took stain of acetocarmine were considered as viable and unstained as non-viable. Pollen grains were observed randomly from average of five microscopic fields and number of viable and non-viable pollen grains were recorded. Total number of stained and unstained pollens were counted and pollen fertility was calculated as the percentage of stained to the total pollen. To estimate pollen germinability, three entire anthers from different flowers on three different plants were harvested, bulked, and immediately placed in the appropriate pollen germination media. Germination of pollen was performed in cavity slides containing 0.5 ml of an aqueous germination solution containing 0.3 M sucrose, 2.43 mM boric acid, and 3 mM calcium nitrate [14]. Pollen germination percentage is calculated as the percentage of number of pollen grains germinated to the total number of pollen grains observed (germinated + ungerminated). Days to 50% flowering was recorded for each plot when 50% of the plants in the plot had 50% of the panicles in anthesis. Two rows, one each of selfed and open pollinated, were harvested seperately. Well dried panicles were weighed to record panicle weight. They were threshed and seeds were separated. Weight of the seeds in grams was recorded. Grain yield was divided by the number of panicles to obtain per plant grain yield. Grain yield per panicle was divided by panicle weight to obtain grain to head weight ratio.

Data analysis

The data was subjected to split plot analysis considering sowing date as main plot and genotype as sub plot. The main plot was tested against main plot error while the genotype and sowing date × genotype interactions were tested against overall error. The relationships between the traits were evaluated by Pearson correlation coefficients. The data was analysed using Genstat 12th edition.

Results and discussion

Analysis of variance

There were non-significant differences among the genotypes and the date of sowing for the average minimum temperature recorded during flowering. This indicates that the temperature differences while recording data on all the genotypes were non-significant. Also, the non-significant temperature mean squares among the sowing dates indicated that similar temperatures prevailed during the two sowing dates (Table 1). However the significant interaction effect of date of sowing with the genotype indicated that individual genotypes were exposed to significantly varying temperatures across the two dates.

Genotypes differed significantly for all the traits denoting sufficient genetic variability existed among the genotypes indicating the suitability of the genetic material for the current study. Date of sowing significantly influenced pollen germinability while it interacted significantly with the genotype in influencing days to 50% flowering, open panicle grain yield and grain to head weight ratio under open pollination. The variance due to sowing date for pollen germinability was more than the variance due to genotypes. Thus the cooler night temperature highly influenced the pollen germinability as compared to the influence of genetic background. Earlier workers observed that pollen performance is affected by the pollen genotype which can lead to rapid pollen tube growth and selection for fixation of this trait. However temperature during the progamic phase affects pollen germination and pollen tube kinetics in the style and this response is genotype dependant [15]. As against pollen germinability, pollen viability is not influenced by sowing date or genotype × sowing date denoting that this trait has less phenotypic plasticity and highly heritable similar to that observed by Lopez *et al.* [12] for pistil traits in sorghum.

Effect of cold stress at anthesis on pollen viability, germinability and agronomic traits

The genetic material was grouped into varieties, maintainer lines, restorer lines and hybrid and presented in Table 2. The varieties showed overall good performance for pollen germinability (91.4%), pollen viability (91.6%) and open panicle grain yield (72.5g/ panicle). Similarly restorer lines also had good pollen germinability (91.9%) and viability (91.9%) but had comparatively less grain yield (58.3g/ panicle) than the varieties (Fig. 1). However the loss of grain yield (open panicle grain yield-selfed panicle grain yield) was about 5g/panicle in varieties while it was 1.9g/panicle in restorer lines. CSV 216R (95.7g/panicle) among the varieties and AKR 354 (73.4) among the restorer lines gave higher open-panicle grain yields in their respective groups.

Among all the genotypes studied, the hybrid CSH 15R (62.1%) and the maintainer line B 35 (64.8%) recorded poor germinability while CSH 15R recorded poor viability (65.8%). The maintainer lines showed comparatively poor germinability (85.3%) though they had good viability (90.8%). The mean open panicle grain yield of B-lines (51.4 g/panicle) was lower compared to varieties and restorers. Low grain yield due to

Table 1. Analysis of variance for pollen and agronomic traits tested in 21 genotypes across two dates of sowing

Source of variation	df	Minimum temperature	Germinability (%)	Viability (%)	Days to 50% flowering	Single panicle weight (g)		Single panicle grain yield (g)		Grain to head weight ratio	
						Open	Self	Open	Self	Open	Self
Date of sowing	1	258.1	550.5**	95.0	107.2*	1564.1	5560.8	389.3	2017.4	0.02	0.01
Error	1	4.7	0.03	204.4	0.3	454.9	264.5	375.6	395	0.004	0.01
Genotype	20	11.4	326.3**	159**	66.9**	1385.7**	1150.6**	1266.5**	953.1**	0.01**	0.02**
Date of sowing x genotype	20	17.4**	137.2	18.4	21.4**	384.6	253.8	340.0*	177.8	0.01*	0.01
Error	40	6.8	85.6	12.2	5.1	207.2	184	153.4	126	0.003	0.01

Table 2. Mean performance of 21 genotypes grouped into four groups for pollen and agronomic traits

Genotype	Germinability (%)	Viability (%)	Days to 50% flowering	Minimum tempe-rature (°C)	Single panicle weight (g)		Single panicle grain yield (g)		Grain to head weight ratio	
					Open	Self	Open	Self	Open	Self
Maintainer lines										
296B	83.0	89.2	91	11.6	56.1	68.7	38.1	48.4	0.7	0.7
104B	88.4	86.2	86	13.0	71.9	78.6	55.1	52.7	0.8	0.7
1409B	82.9	90.5	84	13.6	81.9	78.5	62.3	52.9	0.8	0.6
M31-2B	91.5	92.4	82	16.6	78.9	81.3	60.6	56.4	0.8	0.7
AKRB306	94.9	93.4	79	17.7	76	74.8	64.3	59.1	0.8	0.8
AKMS14B	91.9	94.2	78	11.9	68.1	47.3	49.4	29.8	0.7	0.6
B35	64.8	89.8	74	15.9	41.7	30.8	29.9	20.4	0.7	0.6
Average	**85.3**	**90.8**	**82**	**14.3**	**67.8**	**65.7**	**51.4**	**45.7**	**0.8**	**0.7**
Mean										
M35-1	93.4	93.3	83	14.9	85.9	85.6	68.2	66.2	0.8	0.8
DSV4	92.4	92.7	83	14.5	59.4	76	42.8	55.3	0.7	0.7
CSV216R	90.6	92.1	82	14.6	116.7	109.1	95.7	86.5	0.8	0.8
C43	82.3	82.2	81	14.7	70.2	65.7	53.1	48.5	0.8	0.7
Phule Chitra	93.0	89.8	80	14.9	103.9	94.5	86.4	78.4	0.8	0.8
Parbhani Moti	95.2	93.5	79	15.3	109.9	98.9	94.4	78.1	0.9	0.8
Phule Maulee	94.9	95.1	77	15.1	82.3	82.2	65.6	66.8	0.8	0.8
Phule Anuradha	89.0	94.1	74	16.6	88.9	75.6	73.8	60.5	0.8	0.8
Mean	**91.4**	**91.6**	**80**	**15.1**	**89.7**	**86.0**	**72.5**	**67.5**	**0.8**	**0.8**
Restorer lines										
AKR354	91.7	91.8	81	15.2	88	82.9	73.4	62	0.8	0.
AKR150	89.8	91.6	80	14.9	68.8	55.9	47.7	38.3	0.7	0.7
SLR46	93.3	92.3	79	16.2	76.7	84.2	61.9	68	0.8	0.8
RS585	93.1	93.5	77	16.5	80.6	75.6	64.7	60.1	0.8	0.8
SLR5	91.6	90.3	73	13.2	55.8	67.5	43.8	53.6	0.8	0.8
Mean	**91.9**	**91.9**	**78**	**15.2**	**74.0**	**73.2**	**58.3**	**56.4**	**0.8**	**0.8**
Hybrid										
CSH15R	62.1	65.8	77	11.7	100.8	77.8	82.7	59.8	0.8	0.8
lsd (5%)	13.3	5.0	3	3.7	20.7	19.5	17.8	16.1	0.1	0.1

mid-season cold stress in grain crops has been reported to result in flower abortion, pollen and ovule fertility, failed fertilization, and slow grain fill [16]. However, the loss in grain yield in B-lines was about 5.7g/panicle which is comparative to the varieties though the grain to head weight ratio is reduced by 10%. Earlier reports have shown that low night temperature during flowering causes a reduction in spikelet numbers in rice [17, 18].

Effect of cold stress at anthesis on hybrid and its parental lines

The hybrid CSH 15R showed lower pollen germinability and viability than the other groups. Though the hybrid showed good open panicle grain yield of 82.7g/panicle, the loss in grain yield was about 22.9g. This result points towards realizing the higher grain yields in the hybrid with improvement in the pollen germinability and viability. The pollen viability in the hybrids decreased with increased genetic distance between the parents and when the parents had low pollen viability [19]. The hybrid CSH 15R is based on the cross between 104A and RS 585. The counterpart B-line of the A-line 104A i.e., 104B and RS 585 are included in the current study. The pollen germinability of the maintainer line 104B was 88.4% while for RS

585, it was 93.1%. The open-panicle grain yield of 104B was 55.1g/panicle as against RS 585 with 64.7g/panicle. However, the difference in the grain to head weight ratio of the B-line was low by 0.1 in selfed panicles as against 0 in the R-line which also indicates comparative susceptibility of the B-line. However, there was no difference in the harvest index in the hybrid. As the hybrid has the fertility restorer genes in the nucleus and a sterile cytoplasm, the influence of cytoplasm on pollen germinability needs to be studied in both *kharif* and *rabi* genetic backgrounds.

Trait associations

Germinability and viability were positively and significantly associated with each other and with minimum temperature recorded during pollen collection period i.e., as the temperature increased until optimum, the percentage of viability and germinability also increased. Though the germinability percentage was significantly and positively associated with selfed panicle weight, grain yield and harvest index, the viability percentage did not show such association (Table 3). Poor seed set may be caused due to either or both male and female sterility. In this study, low temperatures caused poor pollen germination especially in the hybrid and B-lines thereby affecting the seed set. However, the correlation with R-lines (R^2=0.83) and B-lines (R^2=0.46) were more linear than varieties (R^2=0.33) (data not shown). This indicates that the hybrid parental lines i.e., R-lines and B-lines are highly responsive to temperatures while the varieties were comparatively less responsive. From this we can infer that the varieties were more tolerant to low temperatures. Most of the *rabi* varieties were developed from the landraces which evolved over the years due to natural selection. This conclusion also supports the hybrid's susceptibility to cold stress being contributed by the parental lines. Hence there is a need to breed for hybrid parental lines with tolerance to cold stress at anthesis or good pollen germinability.

Apart from pollen traits, other associations were also studied. The days to 50% flowering did not associate with any of the traits. The panicle weight, grain yield and grain to head weight ratio of selfed and open pollinated panicles were significantly and positively associated with each other.

The main findings from the study are that the lower seed-set at low temperatures was due to lower pollen germination, though the role of stigma receptivity cannot be completely overruled. The study showed that the germinability of *rabi* sorghum genotypes subjected to cold stress at anthesis is reduced, the

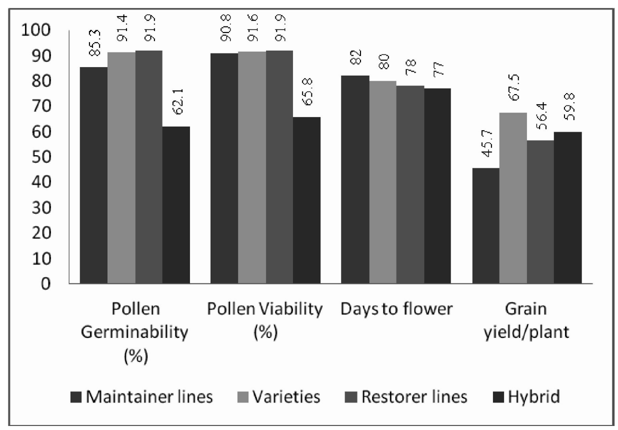

Fig. 1. Performance among groups of sorghum genotypes for pollen viability and germinability and agronomic traits

Table 3. Correlation among the sorghum genotypes for pollen germinability, viability and other agronomic traits

	Germina-bility (%)	Viability (%)	Days to 50% flowering	Minimum tempe-rature (°C)	Grain to head weight ratio (open)	Grain to head weight ratio (self)	Single panicle grain yield (open)	Single panicle weight (open)	Single panicle grain yield (self)
Viability (%)	0.76**	1.00							
Days to 50% flowering	0.12	0.00	1.00						
Minimum temperature	0.36*	0.49*	-0.38	1.00					
Grain to head weight ratio (open)	0.28	-0.05	-0.17	0.31	1.00				
Grain to head weight ratio (self)	0.35*	-0.03	-0.27	0.32	0.60**	1.00			
Single panicle grain yield (open)	0.23	-0.10	-0.09	0.18	0.75**	0.61**	1.00		
Single panicle weight (open)	0.22	-0.11	-0.01	0.12	0.70**	0.55**	0.99**	1.00	
Single panicle grain yield (self)	0.46*	0.08	0.09	0.24	0.73**	0.77**	0.85**	0.83**	1.00
Single panicle weight (self)	0.46*	0.06	0.26	0.15	0.71**	0.65**	0.83**	0.83**	0.97**

intensity varying among the diverse groups of sorghum genotypes. The hybrid was more susceptible to cold stress among all the groups as also seen by the loss in grain yield of 22.9g/panicle. Bringing in tolerance to cold stress in hybrids will ultimately lead to good seed set in the hybrids which will realise their heterotic potential. Though grain yield of R-lines showed linear relationship with pollen germinability, not much loss of grain yield is observed. This points towards good stigma receptivity of R-lines which needs to be studied. The loss in grain yield among both varieties and B-lines was around 5g/ panicle. However the grain yield of B-lines showed good linear relationship with the pollen germinability. It can be inferred that B-lines should be improved for pollen germinability and stigma receptivity studies should be conducted in both varieties and B-lines.

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