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Short Research Article

Pollen Fertility and Seed Setting: Their Role in Deciding the Yield of Sorghum (*Sorghum bicolor* (L) Moench) under Low Temperature Regimes

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Abstract

Experiment was conducted at Regional Agricultural Research Station, Bijapur, India, to understand the role of pollen fertility and seed setting % on the yielding potentiality of sorghum genotypes under low temperature regimes. Experiment was imposed in replicated trials on 35 sorghum genotypes, planted in RCBD design. Each set of experiment was repeated six times with an interval of 7−8 days between the experiments, so as to make peak flowering coincides with lowest possible temperature. Pollen fertility and seed setting behavior of the plant were calculated by standard formula. Correlated the pollen fertility with seed setting along with other yield attributing traits and estimated the effect of low temperature regimes on them. Results indicated that prevailing low temperature has not affected pollen fertility rather than seed set; thereby yield has much affected by low temperature. Association study indicated that pollen fertility did not has significant positive effect on yield and seed setting which plays major role in deciding the yield of the sorghum in low temperature regimes. Among the tested genotypes stay green types were more susceptible to low temperature. Varieties performed better compared to hybrids under given condition indicating their stable performance over the temperature regimes. A maintainer line, M 31-2B which was stable for pollen fertility, seed set percentage as well as yield plant^{-1}, can be used as source for low temperature tolerance in future sorghum breeding program.

1. Introduction

Sorghum is one of the main staple food for the world's poorest and most food insecure people across the semi-arid tropics. Globally, sorghum is cultivated on 41 mha to produce 64.20 mt, with productivity hovering around 1.60 t ha^{-1}. With exceptions in some regions, it is mainly produced and consumed by poor farmers. India contributes about 16% of the world's sorghum production. It is the fifth most important cereal crop in the country. In India, this crop was one of the major cereal staple during 1950's and occupied an area of more than 18 mha but has come down to 7.69 mha. The decline has serious concern on the cropping systems and the food security of these dry land regions of the country. All India total sorghum production has registered a constant growth rate of 0.10% annum^{-1} during the period 1967−68 to 2010−2011 which can be mainly attributed to negative production of *kharif* sorghum rather than positive growth in *rabi* sorghum production. Though, *kharif* sorghum yield growth rates were relatively higher, it could not offset the declining growth rates in production, as the growth rates in *kharif* sorghum area were negative and high. Just opposite is true in case of *rabi* sorghum where the area decline was not sufficient to undermine the yield growth, thus resulting in positive production growth rates. But the overall increase in productivity of *kharif* is far more than *rabi* sorghum (Anonymous, 2014).

In south Asia, particularly in India major high quality sorghum production comes from post-rainy (*rabi*) season crop grown under receding soil moisture after the cessation of the rains with their peak flowering coincides with the cooler days in winter. Low temperature throughout the growing season lengthen the growth cycle of cold susceptible genotypes cause delay in flowering (Gonzalez, 1977), induces the development of sterile pollen, affect pollination and cause scarcity or a total lack of seed in panicles or by poor seed setting (Downes and Marshall, 1971; Ortiz and Carballo, 1972 a, b; Brooking, 1976;

Gonzalez, 1977; Reddy et al., 2012). Cold conditions cause an average annual yield reduction of 5−10% (Angus and Lewin, 1991; Jacobs and Pearson, 1994) in rice.

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 (Reddy et al., 2003). Hence, seed setting ability in hybrids at low temperature is critical to the success of post-rainy season hybrids. This requires greater attention to ascertain the differences among the hybrid parents for their ability to set seeds especially under low temperature.

The improved cultivars derived by involving temperate types are highly productive but relatively photoperiod insensitive and temperature sensitive. Because of this temperature sensitivity, plant growth is reduced and developmental process is delayed resulting in reduced seed set. Among the improved cultivars, hybrid parents and hybrids *per se* are more sensitive to temperature variation than the varieties. Rao (1986) indicated that, if temperature sensitivity is eliminated both in male and female parents, greater success can be achieved in developing hybrids for post-rainy season.

Preliminary information is needed to either select the temperature insensitive genotypes or to find out role/behavior of component traits to decide its worthiness for temperature insensitivity for future crop improvement program. Majority of the earlier reports (Dhopte and Eastin, 1990, 1992; Osuna et al., 2003; Daniel T et al., 2006; Thakur et al., 2010) says that low temperature induces pollen sterility in grain crops. But few studies ruled out the possibility of negative effect of cold temperature on pollen fertility (Sivasubramaniam et al., 1972; Sampath, 1964). Peacock, 1981, has also emphasized the need of further studies on temperature induced pollen sterility in relation to grain yield under field condition. Hence study has been focused to know the role of pollen fertility and seed setting percentage in deciding yield of sorghum under low temperature regimes.

2. Materials and Methods

The experiment was carried out during *rabi* season 2006−07 at Regional Agriculture Research Station, Bijapur with varieties (4), hybrids (7) B-lines (13) R-lines (9) and stay green lines (2). These 35 genotypes were grown in randomized complete block design with two replications. The genotypes were allotted randomly within each replication. Each genotype was grown in two rows of four meter length with 60×15 cm² spacing. Each set of experiment was repeated six times with 7−8 days interval between the experiments so as to make coincides the lowest possible temperature at the time of flowering and seed setting. The six dates of sowing were, a) 20th September, b) 28th September, c) 5th October, d) 12th October, e) 19th October and f) 27th October. The first four dates of sowing were taken under rain-fed situations and the last two dates were given with irrigation at the time of sowing as the moister was insufficient for germination. Recommended package of practices were followed and the crop stand and crop growth were satisfactory in all the six environments. Metrological data for entire crop period starting from sowing of first set of experiment (38th standard week) to harvesting of last set of experiment (8th standard week) was recorded on daily basis.

In each replication and in each genotype five plants were randomly selected for taking observations. Twelve quantitative characters viz., plant height, days to 50% flowering, number of leaves plant^{-1}, length of the panicle, panicle weight, panicle diameter, number of primaries panicle^{-1}, number of grains panicle^{-1}, 500-grain weight, grain yield plant^{-1}, seed setting % and pollen fertility % were recorded. Out of five plants, two plants were tagged and selfed. These plants were used to estimate seed set % as follows,

$$\text{Seed set (\%)} = \frac{\text{No. of filled seeds}}{\text{Total no. of spikelet ear}^{-1}\text{ head}} \times 100$$

For the estimation of pollen fertility, pollen was collected in the morning hours from plant which shows full blooming and pollens were treated with acetocarmine stain and viewed under compound microscope. Pollen which got stained was considered as fertile pollen. Total numbers of stained and unstained pollens (Figure 1) were counted and pollen fertility was calculated by using the formula,

$$\text{Pollen fertility \%} = \frac{\text{Stained pollen}}{\text{Total pollen}} \times 100$$

Data generated on these parameters were analyzed using

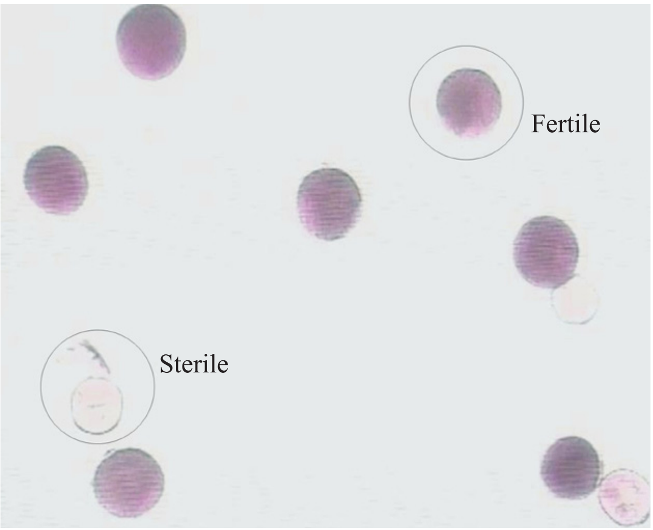

Figure 1: Sterile and fertile pollen grains observed under compound microscope at 100X magnification

Window Stat Version 8 (Indo stat services, Hyderabad, India) statistical software.

3. Results and Discussion

Analysis of variance indicated genotypes under study were significantly differing for each studied traits. Since each genotypes behaved differently in different set of experiment (environment), to know the stable genotypes across the environment, these were tested under Eberhart and Russell (ER) (1966) model for their stability (Table 1). According to the criteria given in ER model, total seventeen genotypes viz., AKMS 14B, M31-2B, BJMS-2B, 27B, 101B, BRJ 204B, SPV-570, BRJ 62, C-43, RS 29, RS 585, BRJ-358, DSV-5, DSV-4, M35-1, BRJ H 129, and CSH-14 found to be stable for pollen fertility. For the seed setting %, twelve genotypes viz., 116B, M31-2B, BJMS-1B, R-354, RS 29, BRJ-358, CSV 216R, DSV-5, DSV-4, DSH-4, BRJ H 129 and CSH 15R were found stable. Similarly for the yield, only three genotypes viz., M31-2B, R-16 and M35-1 appears to be stable. Though some of the genotypes viz., RS 29, BRJ-358, DSV-5, DSV-4 and BRJ H 129 found stable in the production of high fertile pollen and good seed setting behavior but they are not stable grain yielder. Only M31-2B, a maintainer line found to be stable for all the three traits and M35-1, a locally adapted land race found stable for both yield and pollen fertility. From the above table it is clear that genotypes which were found stable for pollen fertility may not be stable for seed setting and yield. This is true for seed setting and yield individually

Table 1: Stability parameters for pollen fertility seed setting and yield

Sl. No.	Genotypes	Pollen fertility (%)			Seed setting (%)			Yield plant^{-1} (g)		
		Mean	bi	S^{2}di	Mean	bi	S^{2}di	Mean	bi	S^{2}di
1.	401B	90.82	0.95	-2.67	65.71	1.70	28.99	40.64	0.86	27.22
2.	104B	92.70	-0.11	15.58	65.35	1.23	-6.88	42.48	1.05	61.11**
3.	296B	88.46	7.08	121.56**	57.70	-1.41	243.71**	29.31	0.19**	-4.50
4.	AKMS 14B	96.21	2.94	0.43	52.92	0.04	252.54**	24.50	0.09**	6.91
5.	1409B	90.80	1.96	33.78**	64.33	0.68	16.47	29.32	0.52**	0.00
6.	116B	94.32	-1.02*	-8.89	82.61	0.42	-7.93	33.70	0.33**	9.32
7.	M31-2B	96.00	0.25	-9.01	77.95	0.85	64.56	46.53	0.80	22.28
8.	BJMS-1B	92.98	-0.24	0.18	77.56	1.35	6.69	33.47	1.05	69.97**
9.	BJMS-2B	93.57	0.30	-8.22	75.60	0.75	146.84*	34.68	0.54**	-3.18
10.	ICSB-37	89.85	3.95**	-10.59	46.47	1.39	392.68**	34.90	0.66	85.84**
11.	27B	95.95	-0.05	-8.14	60.56	1.61	-19.10	24.20	0.57	16.22
12.	101B	94.77	2.37	-1.05	56.70	0.93	281.69**	19.85	0.31**	-16.37
13.	BRJ 204B	97.00	0.80	-13.27	67.38	0.50	-31.97	50.98	1.77	127.20**
14.	SPV-570	97.18	0.20	-10.43	74.78	2.05*	-34.04	49.38	1.67*	40.00*
15.	BRJ 62	95.36	-0.02	10.34	73.95	0.61	136.77*	39.66	0.85	79.91**
16.	R-354	93.22	1.29	32.98**	82.23	0.71	-60.97	50.80	1.73	136.79**
17.	C-43	94.36	1.15	-2.10	64.38	0.82	390.31**	35.90	1.01	17.18
18.	RS 29	96.91	0.53	-11.19	71.71	0.83	-23.88	47.05	1.80*	54.93**
19.	AKR-150	87.53	2.21	19.97	45.24	1.26	1289.29**	28.98	0.77	-1.10
20.	RS 585	96.48	-0.47	-4.73	74.18	0.59	137.67*	54.04	1.34	54.18**
21.	R-16	93.74	1.58	-3.99	59.15	0.28	159.31**	43.57	1.25	0.43
22.	B-35	94.61	-0.61*	-9.36	61.00	2.40	251.36**	26.47	0.81	-4.99
23.	RSG-03123	93.52	2.01	40.23**	49.14	1.10	355.31**	25.79	0.48	137.13**
24.	BRJ-358	97.49	0.59	-11.52	83.66	0.72	-49.34	54.29	1.45	142.96**
25.	CSV 216R	97.48	0.01	-13.46	83.31	0.38	-12.50	58.14	1.62*	24.39
26.	DSV-5	96.72	0.83	-12.64	85.84	1.00	-59.44	49.15	1.68**	-1.29
27.	DSV-4	95.59	0.23	2.02	80.46	0.11	5.54	47.49	1.21	79.54**
28.	M35-1	97.17	0.83	-13.45	67.59	4.08	298.96**	44.92	0.77	1.85

also. To know the exact relation between these parameters along with other yield attributing traits, trait-wise association study (Table 2) has been conducted. Plant height had positive correlation with number of leaves, panicle weight, panicle diameter, number of primaries panicle^{-1}, test weight, number of seeds and yield plant^{-1}. It clearly says that as the height of the plant increases it will positively supports the other yield attributing traits to produce finally yield by converting biomass in to economic yield. Number of leaves associated positively with number of primaries panicle^{-1}, pollen fertility, test weight and yield plant^{-1}. Panicle weight, panicle diameter, number of primaries panicle^{-1}, number of seeds and yield had positive inter correlation among them. Number of primaries panicle^{-1} and panicle weight had positive association with seed setting. As expected test weight and seed setting % had positive correlation with yield. It indicated, if other things remains constant higher seed set and test weight influences yield in positive direction. Pollen fertility has shown poor and non-significant positive correlation with yield plant^{-1}. It may be due to higher proportion of cross pollination observed in some cold susceptible genotypes, under low temperature condition during the pre-boot stage (Osuna et al., 2003) in sorghum. Hence genotype with good and stable pollen fertility may not be a high yielding one but we can select genotype for high yield based on its seed setting behavior.

One of our objectives was to test whether prevailing temperature has any role on physiological changes viz., changes in duration for maturity, ability to produce fertile pollen and seed set. We planned and plant the experiment in such a way that every set of experiment should expose to different temperature regimes at the time of sowing to flowering. Genotypes grown after 12th October onwards were exposed to lowest (approx. 10 °C) temperature for the period of three weeks which exactly coincides with peak flowering period. As temperature goes below 10 °C during reproductive stage, the flowering was delayed up to 78.48 days and seed setting was reduced as low as 57.79% (Table 3). Gonzalez, 1977; Osuna et al., 2003 observed delayed flowering due to low temperature and negative effect of low temperature on stigma receptivity results in low seed setting, respectively. Optimum temperature for sorghum growth is 30 °C; however, 13 °C is the critical low temperature during reproductive development (Downes and Marshal, 1971). Low night temperature during leptotene in meiosis can adversely affect microspore development, resulting in male sterility and infertile pollen formation (Brooking, 1976; 1979). However, as yet, there is no unequivocal evidence for the occurrence of low temperature-induced pollen sterility in sorghum and further detailed field work is required to elucidate the potential importance of this type of sterility induction (Peacock, 1981). Interestingly in present study, pollen fertility was not significantly affected by the lower temperature and it recorded more than 90% fertility in genotypes grown in all six dates. This observation is in contrary to the earlier report by Downes and Marshall., 1971, Ortiz and Carballo, 1972 a, b; Brooking, 1976; Gonzalez, 1977; Reddy et al., 2012 where they indicated cold induced pollen sterility was the major reason for reduced seed set. But in present study pollens appear to be fertile in all the genotypes. Because pollens collected from the test plant were immediately (within 1 hour) observed under microscope for its viability; hence they may not show sterility. But as soon as it detached from the anther sac and before settling on stigmatic surface, it may get cold shock and

Table 2: Correlation among the yield and yield component traits in sorghum

Characters	NLS	D 50% F	PNWT	PNLT	PNDIA	NP	PF	SS	TW	NS	YPP
Plant height (PH)	0.59^{**}	0.04	0.50^{**}	-0.22	0.47^{**}	0.57^{**}	0.28	0.31	0.44^{**}	0.44^{**}	0.60^{**}
No. of leaves (NLS)		0.01	0.18	-0.19	0.29	0.30^{*}	0.37^{*}	0.27	0.36^{*}	0.25	0.40^{*}
Days to 50% flowering (D 50% F)			0.12	-0.44^{**}	0.21	0.18	0.54^{**}	0.50^{**}	-0.47^{**}	0.31	0.06
Panicle weight (PNWT)				0.06	0.53^{**}	0.73^{**}	0.18	0.42^{*}	0.14	0.70^{**}	0.71^{**}
Panicle length (PNLT)					-0.04	0.05	0.32	0.07	-0.21	-0.12	-0.20
Panicle diameter (PNDIA)						0.49^{**}	0.10	0.30	0.36^{*}	0.46^{**}	0.56^{**}
No. of primaries panicle^{-1} (NP)							0.14	0.51^{**}	0.20	0.63^{**}	0.66^{**}
Pollen fertility (PF)								0.23	0.03	0.27	0.25
Seed setting (%) (SS)									-0.03	0.44^{**}	0.39^{*}
500 seed weight (TW)										-0.06	0.38^{*}
No. of seeds (NS)											0.86^{**}

*Significant at (p=0.05) probability level; **Significant at (p=0.01) probability level

Table 3. Character means, range and prevailing temperature at the time of sowing and flowering

Sowing dates	Range of days to 50% flowering		Pollen fertility %		Seed set %		Temperature in different mean standard week													
							Temperature at sowing						Temperature at flowering							
	Mean	Range	Mean	Range	Mean	Range	38	39	40	41	42	43	48	49	50	51	52	1	2	3
1st date	77.48	73.00-82.5	91.18	58.60-97.50	65.40	36.50-86.00	20.90	-	-	-	-	-	17.00-30.70	13.10-29.70	13.30-29.80	-	-	-	-	-
2nd date	72.17	65.00-80.50	93.87	85.50-98.90	74.12	31.40-92.80	-	20.80	-	-	-	-	17.00-30.70	13.10-29.70	13.30-29.80	11.10-28.70	-	-	-	-
3rd date	67.98	62.500-74.50	92.70	72.60-98.40	70.94	24.90-95.10	-	-	21.10	-	-	-	-	13.10-27-70	13.30-29.80	11.10-28.70	-	-	-	-
4th date	68.98	59.00-79.50	93.05	81.20-97.90	73.77	6.40-95.50	-	-	-	20.10	-	-	-	-	13.30-29.80	11.10-28.70	10.70-29.50	-	-	-
5th date	71.37	66.00-79.50	94.41	79.50-99.00	64.85	9.10-90.80	-	-	-	-	19.50	-	-	-	-	-	10.70-29.50	9.90-29.30	-	-
6th date	78.48	69.00-86.50	95.35	82.60-99.70	57.79	17.00-79.00	-	-	-	-	-	18.20	-	-	-	-	-	9.90-29.30	11.20-29.60	12.80-31.40

1st date -20th September; 2nd date -28th September; 3rd date -5th October; 4th date -12th October; 5th date -19th October; 6th date -27th October

may get unviable. Other possible cause could be cold induced pre-fertilization barrier which makes stigmatic surface to resist the pollen tube growth, there-by inability to fertilize the ovary causing no seed set. Srinivasan et al., 1999 also observed reduced another dehiscence due to low temperature resulting in low pollen load on stigma causing reduced pollen transfer thus limiting fertilization in chickpea.

Yield component traits are compared among the B lines, R lines, stay green lines, varieties and Hybrids (Table 4). In all six set of experiments, hybrids matured relatively early compared to other genotypes. Panicle weight of varieties (63.4 g) were high compared to hybrids (61.60 g) followed by R-lines, B-lines and Stay green lines. Similarly varieties showed profuse branching of panicles as indicated by more mean number of primaries panicle^{-1} (58.0) compared to hybrids (55.80 g) followed by R-lines, B-lines and Stay green lines. Hybrids showed more sensitivity for the production of fertile pollens (89.90%) under cold conditions compared to other set of genotypes. Seed setting was drastically reduced (55.10%) and thereby yield (26.10 g plant^{-1}) in two stay green genotypes, B35 (temperate type) and RSG03123 (derivative of temperate´tropical cross). Due to the presence of temperate blood in these genotypes, growth and developmental process is negatively affected (Rao, 1986). Varieties recorded high pollen

Table 4: Comparative mean performances of maintainers, restorers, stay green lines, varieties and hybrids for different characters

Sl. no.	Genotypes	PH	NL	D 50% F	PW (g)	PL (cm)	PD (cm)	NPP	PF (%)	SS (%)	500 SW (g)	NSP	YP (g)
1.	B lines (13)	131.60	6.50	72.50	46.90	21.80	12.40	50.60	93.30	65.40	13.50	1261.60	34.20
2.	R line (9)	144.90	7.50	73.90	54.80	18.50	13.30	52.50	94.70	69.90	15.20	1465.50	44.90
3.	Stay green lines (2)	108.60	7.10	72.30	38.80	20.10	11.90	45.90	94.10	55.10	14.20	923.70	26.10
4.	Varieties (4)	190.40	8.20	76.70	63.40	19.40	14.10	58.00	96.70	79.30	16.70	1530.00	49.90
5.	Hybrids (7)	163.80	7.10	69.60	61.60	22.70	13.30	55.80	89.90	66.60	15.30	1583.50	49.40

PH: Plant height; NL: No. of leaves; D 50% F: Days to 50% flowering; PW: Panicle weight; PL: Panicle length; PD: Panicle diameter; NPP: No. of primaries panicle^{-1}; PF: Pollen fertility; SS: Seed set; SW: seed weight; NSP: No. of seeds panicle^{-1}; YP: Yield plant^{-1}

fertility (96.70%) as well as seed set (79.30%) even compared to hybrids. Because of their topical origin they found less sensitive to change in temperature regimes and better adapted to prevailing conditions. But yield of varieties and hybrids were matching each other due to the increased panicle length (22.70 cm) as well as mean number of seeds panicle^{-1} in hybrids (1583.50) in comparison with variety.

4. Conclusion

The seed set % is the major deciding factor of yield potentiality of the genotypes. M 31-2B, appeared as stable genotype for pollen fertility, seed setting and yield, could be used as source for the identification of QTL for low temperature tolerance in sorghum by developing mapping population using them in a cross combination with susceptible genotype (B 35 or RSG 03123). Pre-fertilization barriers should be studied to find out the exact reason for reduced seed set in sorghum.

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