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Swaroop KO
Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad, Karnataka, India

Sajjanar GM
Regional Agricultural Research Station, Vijayapur, University of Agricultural Sciences, Dharwad, Karnataka, India

Sneha SP
Department of Genetics and Plant Breeding, College of Agriculture, Vijayapura, University of Agricultural Sciences, Dharwad, Karnataka, India

Kiran BO
Department of Plant Physiology, University of Agricultural Sciences, Dharwad, Karnataka, India

Soregaon CD
Department of Genetics and Plant Breeding, College of Agriculture, Vijayapura, University of Agricultural Sciences, Dharwad, Karnataka, India

Corresponding Author:
Swaroop KO
Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad, Karnataka, India

Response of hybrid parents of sorghum during reproductive phase under varied temperature regimes

Swaroop KO, Sajjanar GM, Sneha SP, Kiran BO and Soregaon CD

Abstract
The present investigation was carried out during *rabi* season 2021–22 with 32 elite sorghum genotypes including hybrid parents and check varieties to study their response under different temperature regimes on seed set percentage and pollen fertility percentage. The experimental material was evaluated over four dates of sowing (September-November), at an interval of fortnight as to adjust flowering at different temperature regimes. The experiment was laid out in a randomized block design with two replications. The study revealed that the range of pollen fertility percentage was higher than the range of seed set percentage in all the dates of sowing and temperature regimes. In other words, seed set percentage is more affected by temperature at all the four dates compared to pollen fertility. In general, seed setting percentage was higher in both first and second dates (>90%) compared to third and fourth dates. The effect of sowing dates on different phenotypic characters also revealed that delayed sowing, *i.e.*, on the third and fourth dates of sowing, reduced the expression of all traits studied. In terms of grain yield per plant, the second date (October first week) sown plants yielded more than the remaining dates. Comparative mean performance of B lines for various reproductive traits across different dates with that of R lines, revealed lower mean performance of B lines for all the reproductive characters except grain number.

Keywords: Temperature regimes, seed set percentage, pollen fertility percentage, rabi sorghum, hybrid parents, maintainers (B) and restorer (R)

Introduction
Grain sorghum [*Sorghum bicolor* (L.) Moench] is one of the five most significant cereal crops grown worldwide. It is the third cereal crop grown in India after rice and wheat. It is primarily grown in semiarid tropics and subtropics. In many developing Asian and African nations, grain sorghum is a staple crop for human consumption.

Sowing of post rainy season sorghum in India starts from first week of September and extends up to first week of November depending upon the rainfall pattern. This season is characterized by reduced sunshine hours and cooler nights which affect the crop growth and productivity. Sorghum is a short-day plant, and variation in the response to photoperiod and temperature determines its adaptation to the wide range of environments in which it is grown (Craufurd *et al*. 1999) [4]. So, it is important to know the behavioral genetics of sorghum response to varying day length and temperature regimes. As sorghum originated near the equator in northeastern Africa, it is sensitive to day length and temperature (Miller 1982) [6].

Cold stress affects both vegetative and reproductive phases of the plant life cycle, with the latter being particularly susceptible (Nishiyama 1995) [9]. The reproductive phase begins with transformation of the meristem into inflorescence and flower and, in annuals, ends upon seed reaching maturity. The reproductive phase can be divided into sequential steps: flower initiation, differentiation of male and female floral parts, micro- and mega- sporogenesis, development of male and female gametophytes (pollen grain and embryo sac), pollination, micro- and mega-gametogenesis, fertilization and seed development. All these stages respond differently to cold stress (Staggenborg and Vanderlip 1996; Verheul *et al*. 1996) [13, 14] but collectively all responses are negative and reduce net yield. Low temperature stress during reproductive development induces flower abscission, pollen sterility, pollen tube distortion, ovule abortion and reduced fruit set, which ultimately lowers the yield. Thus, cold stress during the reproductive phase has important economic and social consequences because the reproductive phase products are the key components of economic yield and are the principal source of food for entire humanity.

The improved cultivars derived by involving temperate types are highly productive but relatively photoperiod insensitive and temperature sensitive. Because of this temperature sensitivity, the growth is reduced, development is delayed and seed setting is affected. Among the improved cultivars, hybrid parents and hybrids *per se* are more sensitive to temperature variation than the varieties. 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. If temperature sensitivity is eliminated both in male and female parents, greater success can be achieved in developing hybrids for post-rainy season.

In sorghum, there is variation in seed setting behaviour and response to reduced minimum temperatures among genotypes (Reddy *et al*. 2003) [12]. The study on stability of seed setting behaviour across different temperature regimes in the parental lines, varieties and hybrids will help the breeder to choose appropriate genotypes for the development of hybrids or involving them in crossing programme to generate new breeding material. Furthermore, the range of variability, heritability and genetic gain associated with the traits determine the success of phenotypic selection. Understanding the nature of association of the characters, cause and effect relationship will help indirect improvement through selection for component traits. This will also help to study the behaviour of different genotypes at varied day length periods. This in turn helps in identification of photoperiod and temperature insensitive genotypes for the development of hybrids and varieties for early and delayed sowing dates to tackle the problem of pest in early sowing and drought in delay sowing. Very limited work has been carried out on this aspect in *rabi* sorghum. Keeping these things in view the present study was conducted.

Materials and Methods

The present investigation was carried out during *rabi* season 2021–22 at the Regional Agricultural Research Station, Vijayapur. The experimental material consisted of 32 elite sorghum genotypes including hybrid parents, (restorers or R lines and maintainers or B lines on *milo* and *maldandi* sources of male sterility) and check varieties. The experimental material was evaluated over four dates of sowing (Table 1), at an interval of fortnight as to adjust flowering at different temperature regimes during *rabi* 2021. The experiment was laid out in a randomized block design with two replications. Each line consists of two rows of three meters each, spaced 60 cm apart. All recommended agronomic and plant protection practices were followed regularly as per needed for better crop stand and expression. Observations on panicle weight (g), grain yield per plant (g/plant), seed set percentage, pollen fertility percentage and 100 seed weight (g) were recorded on randomly chosen five plants in each entry. Days to fifty percent flowering was recorded on plot basis. Seed set percentage and pollen fertility percentage were calculated using following formula.

Seed set percentage

To work out seed set percentage, panicles were selfed to avoid the cross pollination. Panicles were counted for total number of unfilled spikelets and total number of seeds per panicle. Seed set percentage was calculated using the formula (Biradar 1995) [2].

$$\text{Seed set \%} = \frac{\text{Total number of seeds}}{\text{Total number of spikelets}} \times 100$$

Pollen fertility (%)

For the estimation of pollen fertility, pollen was collected in the morning hours from plant which shows full blooming, and the sterility percent was observed under microscope using 2.0 percent acetocarmine stain (0.5 g carmine, 55 ml of distilled water and 45 ml of acetic acid which was boiled gently for 5.0 minutes and filtered). Pollen which got stained was considered as fertile pollen. Total number of stained and unstained pollens were counted and pollen fertility was calculated by using the formula (Alexander 1969) [1].

$$\text{Pollen fertility \%} = \frac{\text{Number of stained pollens}}{\text{Total number of pollens}} \times 100$$

Results and Discussion

Low temperature vs seed set and pollen fertility percentage: Stephens and Holland reported a relationship between viable pollen production or pollen shedding and temperature as early as 1954. More specifically, hybrids that are normally male fertile exhibit male sterility when the minimum temperature falls below 10 ºC for several days during flowering (Reddy *et al*. 2003) [12]. In this context, a comparison was made with pollen fertility percentage and seed set percentage *vs* minimum temperature at flowering (Table 1).

The study revealed that the range of pollen fertility percentage was higher than the range of seed set percentage in all the dates of sowing and temperature regimes. Mukri *et al.* (2010) [8] also observed similar results. In other words, seed set percentage is more affected by temperature at all the four dates compared to pollen fertility.

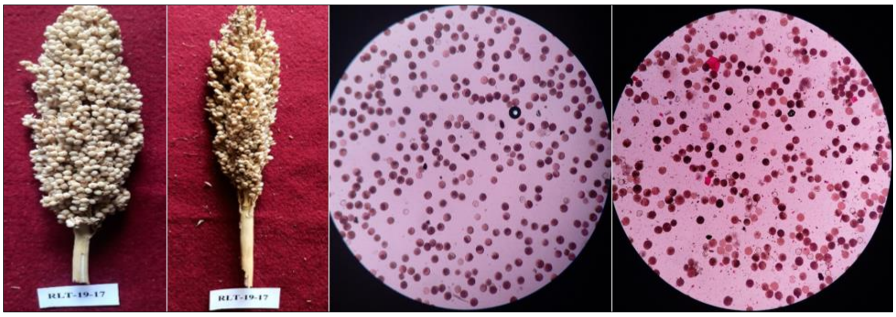

Fig 1: Panicle of RLT-19-17 line at 2nd and 4th date of sowing and sterile and fertile pollen grains of the same genotype at 2nd and 4th date of sowing observed under compound microscope at 10X magnification

This indicates that seed set may not be entirely dependent on the pollen fertility. There could be factors other than pollen fertility influencing seed set percentage. The pre-fertilization stages like stigma receptivity, pollen germination, pollen tube elongation, *etc*., might be sensitive to lower minimum temperature resulting reduced seed set.

The lowest mean pollen viability percentage (95.59%) was observed on the third date of sowing, when the minimum temperature of 9.6 ºC coincided with the flowering period. McLaren (1997) [5] also noticed that pollen viability was significantly reduced by pre-flowering daily minimum temperatures less than 16 °C during the period 23–27 days before flowering in sorghum. Low temperatures influenced the quality and quantity of pollen produced, as well as possibly modifying stigma receptivity (Osuna *et al*., 2003) [10]. The highest range of pollen viability (91.76-100) was noticed with highest range of average minimum temperature (13.5 ºC -21 ºC) in first date of sowing and the lowest range of pollen viability (87.27-100) was noticed with lowest range of average minimum temperature (9.6 ºC-16.8 ºC) in third date of sowing. Until the minimum temperature was above 10 ºC, the range with minimum pollen viability percentage was above 90%.

In general seed setting percentage was higher in both first and second dates (>90%) compared to third and fourth dates (82.64% and 69.27%, respectively). Average minimum temperature of 15.5 °C and average maximum temperature of 32.6 °C was observed during flowering period of fourth date of sowing (Table 1). Although average minimum temperature was higher in fourth date (15.5 °C) compared to second (13.83 °C), indicating daily minimum temperature might have affected seed set percentage in some genotypes resulting in lowest mean seed set percentage. Hence, the range of maximum and minimum temperatures during the reproductive phase of flowering may have influenced seed set percentage, and pollen viability, which is affected by daily minimum temperature, may also have contributed to it. Chaithra *et al.* (2017) [3] reported similar findings. Patel *et al*. (2019) [11] also observed comparable results.

Influence of sowing dates on reproductive traits

In general flowering was early in DoS1 (25th Sept) and DoS2 (8th Oct) and late in DoS3 and DoS4 (Table 1). Mukri *et al.* (2014) [7] also reported that flowering was early when genotypes were sown at 5th October. Also range of flowering time (From-to) among genotypes was varied across different dates of sowing (Table 1).

Table 1: Range values of temperature during flowering period, seed setting (%) and pollen viability (%) across different dates of sowing of B & R lines of *rabi* Sorghum during *Rabi* 2021-2022 at RARS Vijayapur

Sl. No.	Date of sowing	Flowering dates (From - to)	Average temperature (ºC)		Range of temperature n(ºC)		Seed setting (%)		Pollen viability (%)	
			Avg. Max.	Avg. Min.	Max.	Avg. Min.	Mean	Range	Mean	Range
1	25/09/2021	Nov 26-Dec 30	28.65	17.43	25.5-31	13.5-21	90.83	60.50-99.23	97.37	91.76-100
2	08/10/2021	Dec 9- Dec 30	28.96	13.83	27-31.5	10-17.5	92.81	70.88-99.47	97.21	89.18-99.52
3	30/10/2021	Jan 9- Jan 31	29.1	13.3	25.6-32.2	9.6-16.8	82.64	38.78-98.58	95.59	87.27-100
4	08/11/2021	Feb 4- Feb 21	32.6	15.5	28.6-33.5	10.4-17.4	69.27	8.57-97.85	96.43	89.77-99.65

The duration is maximum in 1st date (34 days) and reduced at remaining three dates with minimum at 4th date of sowing in Nov 8th (17 days). Yield per plant was also higher in DoS1 and DoS2 dates and reduced in both DoS3 and DoS4. The trend is same for all the remaining reproductive traits *viz*., panicle weight, 100 seed weight, grain number per plant and panicle harvest index. This indicated that delayed sowing affects reproductive parameters. And September last week and October first week are found to be optimum for sowing to get higher yields. Mukri *et al*. (2014) [7] reported that the grain yield in general was high in the genotype sown at 20-28th September compared to the remaining dates.

Comparative performance of B and R lines

Mean performance of B lines for various reproductive traits across different dates was compared with that of R lines (Table 2). It is observed that mean performance of R lines for all the reproductive characters (seed setting percentage, pollen viability percentage, days to 50% flowering, grain yield per plant, panicle weight, 100 seed weight, and panicle harvest index) is greater than B lines, except for grain number per plant. The results suggested the need to improve B lines for various reproductive characters. Similar results on lowest mean performance of B lines compared to R lines was reported by Mukri *et al*. (2014) [7] for the seed set percentage, test weight and grain number per panicle.

Among fourteen B (maintainer) lines evaluated, the genotypes that recorded narrow range for more number of traits compared to maximum range recorded in BJMS 2B for all the traits across different sowing dates and temperature regimes were *viz*., BLT-I-1, BLT-2010, BJMS 1B, CSV 14RB, M 31-2B and 401B (Table 3). Among eighteen R (restorer) lines evaluated, the genotypes that recorded narrow range for more number of traits compared to maximum range recorded in RLT-19-7 for all the traits across different sowing dates and temperature regimes were *viz*., RLT 2004, RLT 2013, RL 2036, ICSR13039, RS 29, BRJ 62 and C43 (Table 4). Similar results on narrow range of values for various traits across different sowing dates in which the genotypes including maintainer and restorer genotypes that are less sensitive to varying temperature regimes were reported by Mukri *et al*. (2014) [7].

The results on performance of hybrid parents yield related traits across different sowing dates suggest that these genotypes could be used in development of less sensitive hybrid to low temperature using counterpart A lines that are available, and also above listed genotypes may be used further for the development of new B and R lines which are less sensitive to sowing dates and temperature regimes in *rabi* season.

Table 2: Comparative mean performance of maintainers and restorers for different characters across four dates of sowing *Rabi* season

	Seed setting (%)	Pollen viability (%)	Days to 50% flowering	Grain yield per plant (g)	Panicle weight (g)	100 seed weight (g)	Grain number per plant (no.)	Panicle Harvest Index (%)
B lines								
Mean	82.8	96.4	78.7	29.9	44.4	3.3	953.7	65.0
Range	62.3-95.8	94.2-98.1	68.5-95.4	17.9-44.7	29-61.2	2.8-3.8	580.1-1357.6	51.6-73.3
R lines								
Mean	84.75	96.9	80.1	31.5	45.8	3.4	939.4	67.0
Range	65.65-95.78	94.5-98.8	70.6-95.1	18.2-47.2	29.9-63.1	2.9-4.0	552.3-1319.8	54.1-76.2

Table 3: Mean and range for different reproductive parameters among maintainer lines of sorghum that recorded narrow range of values across different dates of sowing during *Rabi* season

Characters	Range & Mean	Narrow range							Max. range
		BLT-I-1	BLT-2010	BJMS 1B	BJMS 3B	CSV 14RB	M31-2B	104 B	BJMS 2B
Seed setting (%)	Range	50.97-93.48	68.77-96.97	72.59-97.23	67.45-92	73.8-94.37	68.29-94.38	88.3-93.52	22.09-95.7
	Mean	80.22	88.43	90.69	80.61	84.66	83.67	91.82	61.84
Pollen viability (%)	Range	96.8-98.13	95.68-98.46	94.48-98.6	93.35-96.7	94.41-96.7	96.63-98.3	92.4-99.3	93.01-97.9
	Mean	97.81	97.02	96.62	95.18	97.37	97.37	96.54	95.28
Days to 50% flowering	Range	65-89	72-97	63-89	67-96	68-91	73.5-93	66-90	65.5-94
	Mean	73.25	80.75	73	77.38	76.13	80.38	75.25	75.5
Grain yield per plant(g)	Range	19.7-33.9	25.5-49	23-40.2	17.2-31.4	19.7-33.8	16-35.8	22.3-30.2	3-36.3
	Mean	27.15	35.6	33.08	24.44	27.21	24.81	26.17	18.2
Panicle weight (g)	Range	27-45.7	34.2-59.5	45-58.3	31.7-47.2	31-49.5	22.3-47.2	36.3-49.3	12-53.2
	Mean	37.25	47.5	52.58	38.54	40.63	34.83	43.33	35.13
100 seed weight (g)	Range	3.42-4.44	2.5-3.45	2.81-2.97	2.83-3.9	3.1-4.08	3.38-4.05	2.62-3.48	2.02-4.1
	Mean	3.94	3.02	2.82	3.29	3.51	3.52	3	3.05
Grain number per plant(no.)	Range	556-775	735-1564	775-1483	538-777	639-920	450-884	759-1047	98-1212
	Mean	688.12	1239.34	1198.42	730.75	784.81	688.43	901.5	612.93
Panicle Harvest Index (%)	Range	69.9-74.73	68.9-82.19	43.4-71.1	54.29-66.9	61.9-68.52	53.1-76.4	58.12-60.76	10-66.3
	Mean	72.89	73.82	62.93	62.59	66.11	69.14	59.99	43.69

Table 4: Mean and range for different reproductive parameters among restorer lines of sorghum that recorded narrow range of values across different dates of sowing during *Rabi* season

Characters	Range & Mean	Narrow range							Max. range
		RLT 2004	RLT 2013	RLT 2036	ICSR13039	RS 29	BRJ 62	C43	RLT-19-17
Seed setting (%)	Range	74.96-96.55	74.9-96.15	72.79-95.97	55.94-97.15	83.58-97.75	85.81-94.4	90.4-95.49	37.01-95.48
	Mean	81.82	88.13	88.38	84.64	92.78	91.50	93.54	75.90
Pollen viability (%)	Range	95.2-99.41	92-97.65	95.5-100	97.3-100	96.18-97.79	95.22-99.7	93.72-96.6	87.9-97.6
	Mean	96.52	95.09	97.46	98.53	97.09	98.07	95.31	92.90
Days to 50% flowering	Range	64-93	73-96	73-92	73-95	63-94	65-91	71-101	71-97
	Mean	76.13	82	81	82	72.75	75.38	83.5	80
Grain yield per plant(g)	Range	18.70-41.70	16.2-44.5	17.6-32.8	23.3-36.3	13.2-26	27.3-42.5	27.5-48.3	8-48.5
	Mean	27.06	27.88	23.7	29.27	20.29	35.5	34.83	32.5
Panicle weight (g)	Range	31-53.7	20.8-58	24.7-42.5	35.5-53.8	22-33.8	39.5-57.8	38-63	29.8-65
	Mean	39.04	40.17	32.79	44.5	28.69	47.58	50.5	48.77
100 seed weight (g)	Range	2.71-3.67	2.85-4.23	2.78-3.49	2.84-3.57	3.37-4.05	2.94-4.08	2.53-3.89	2.57-4.55
	Mean	3.28	3.55	3.08	3.01	3.61	3.46	3.27	3.82
Grain number per plant(no.)	Range	627-1168	432-1043	559-918	744-1184	326-685	669-1214	752-1426	319-1094
	Mean	828.85	794.59	769.41	975.82	569.67	1039.98	1089.62	803.19
Panicle Harvest Index (%)	Range	60.36-77.6	59.02-79.07	65.57-76.1	57.3-67.53	59.67-76.6	69.52-78.6	57.41-77.2	27-77.27
	Mean	67.62	69.78	71.57	64.19	69.45	74.45	68.75	62.9

Conflict of Interest: None

Author's contribution: SKO carried out research, processing and analysis of research data and writing of this manuscript. SSP involved in data recording and analysis. GMS involved in finalization and implementation of research programme, technical guidance and preparation of manuscript of research work. KBO and SCD involved in finalization of research topic, technical guidance and corrected this manuscript.

Conclusion

The study demonstrates that temperature has a greater impact on seed set percentage compared to pollen fertility across all sowing dates. Delayed sowing significantly diminishes the expression of studied traits, indicating reduced performance in later sowing dates. Additionally, while the second sowing date resulted in the highest grain yield per plant, B lines generally exhibited lower mean performance in reproductive traits compared to R lines, except for grain number across all dates of sowing.

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