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Differential Expression of Restorer Gene on Different Nuclear Background with *Maldandi cytoplasm* in Sorghum (*Sorghum bicolor* (L.) Moench)

Pavan Kumar N [a*], B. D. Biradar [b++], N G Hanamaratti [a], Nethra P [c], Prashant Kariyannanavar [a], Revannasiddayya [d] and Prajwal R S [e]

[a] Department of Genetics and Plant Breeding, UAS Dharwad, Karnataka, 580005, India.
[b] University of Agricultural Sciences, Dharwad, Karnataka-580005, India.
[c] Department of Crop Physiology, UAS Dharwad, Karnataka-580005, India.
[d] Department of Genetics and Plant Breeding, UAS Raichur, Karnataka-584104, India.
[e] Department of Genetics and Plant Breeding, UAS Bangalore, Karnataka-584104, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author BDB Research conceptualized, Authors BDB and NGH did Experiment design. Author PKN did Field, lab work and data collection. Authors PKN, BDB and NGH did Data analysis and interpretation. Author PKN did Manuscript drafting. Authors BDB, NGH and RS did Manuscript revision. All authors read and approved the final manuscript.

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ABSTRACT

To investigate the expression of restorer gene on different nuclear background an experiment was carried out using six iso-plasmic male sterile lines with maldandi cytoplasm, and each having different nuclear background. These lines were hybridized with a strong and stable restorer to generate six iso-plasmic hybrids at Main Agricultural Research Station (MARS), UAS Dharwad during rabi -2020-21. These hybrids were then evaluated for pollen fertility and seed set percentage

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[++] Director of Research;
**Corresponding author: E-mail: pavan.n.kumar21@gmail.com;*

during rabi -2021-22. The results found that, pollen fertility percentage of six iso-plasmic hybrids ranged from 69.82 per cent for ICSA 88004 A4 (M) × DSMR 8 to 92.42 per cent for M31-2A (M) × DSMR 8. Similarly, for seed set percentage M31-2A (M) × DSMR 8 and ICSA 88004 A4 (M) × DSMR 8 exhibited highest (85.17 per cent) and lowest (61.19 per cent), respectively. All the six iso-plasmic hybrids had varied expression for pollen fertility and seed set percentage which suggests that the restorer gene expressed differentially in different nuclear genome of female parent. The probable reasons for variation in fertility restoration behaviour are, abundance of inhibitors in the female parent or variation in the expressivity of restorer gene or influence of minor or modifier genes in the restorer parent or interaction between nuclear genes of both the parents.

Keywords: Fertility restoration; Iso-plasmic hybrids; Maldandi cytoplasm; pollen fertility; restorer line; sorghum.

1. INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is the fifth most important cereal crop globally. It is recognized as a nutritious grain millet since, it offers high levels of fiber, protein, calcium, phosphorous and potassium compared to wheat and rice [1,2]. Beyond its role as a staple food crop, sorghum is cultivated for bioethanol, alcohol and fuel production.

Enhancing yield stands as a primary objective of breeding program, with hybrid development emerging as a promising strategy to achieve this objective. The cytoplasmic-genetic male sterility (CGMS) has been utilized in the commercial production of hybrid seeds across a wide range of crops including sorghum [3,4]. Male sterility gene is reported to be associated with chimeric mitochondrial *orf* [5]. The sterility observed in plants with CMS cytoplasm is a result of the influence of CMS proteins on mitochondria. This influence leads to an increase in reactive oxygen species (ROS) [6], initiation of abnormal programmed cell death (PCD) [7], retrograde regulation of nuclear genes [8] and the toxic effects [9,10]. Such process results in production of either non-viable or underdeveloped pollen grains or non-dehiscent anthers with or without functional pollen grains [11]. This approach prevents the laborious manual emasculation and physical injury to floral reproductive organ in the female parent [12]. To overcome male sterility, the male parent harboring dominant nuclear-fertility restorer (*Rf*) gene encode a protein which interact with CMS gene products. This interaction alleviates or eliminate the adverse effects of CMS gene products at DNA [13], transcription [14], post-transcription [15], translation [16-18], post-translation [19], and metabolic [20] levels which ultimately results in the production of fertile F_1 plants. However, the fertility restoration through the action of *Rf* gene(s) of the pollen parent alone seems untenable because it is regulated by the nuclear and cytoplasmic genome interaction. Earlier efforts have shown that the same pollen parent can restore fertility differently on various allo-nuclear male sterile lines *i.e.,* lines having same cytoplasm with different nuclear background [21]. Hence, this variation could be attributed to the genotype of the male sterile parent or by a nucleo-cytoplasmic interaction. These phenomena have been extensively studied in rice. In case of sorghum, similar studies have been conducted using a different CMS-inducing cytoplasm other than *maldandi* (A_4) male sterile source [22]. Hence, this current study was focused to understand the interaction effect of male sterile cytoplasm with nuclear gene for pollen fertility and restoration behavior using iso-plasmic and allonuclear lines having *maldandi* male sterile cytoplasm.

2. MATERIALS AND METHODS

2.1 Experimental Materials

Six iso-plasmic and allo-nuclear male sterile lines having *maldandi* cytoplasm and one strong restorer were utilized in the current experiment (Table 1).

2.2 Production of Iso-Plasmic Hybrids and its Evaluation

During *rabi* 2020-21, restorer (DSMR 8) crossed with the *maldandi* based six iso-plasmic male sterile lines to generate six iso-plasmic hybrids by taking 2 staggered sowings of male sterile line with one-week interval.

The non-replicated trial for evaluation of pollen fertility and seed set percentage of six iso-plasmic hybrids were laid out at Main Agricultural Research Station, UAS Dharwad in *rabi* 2021-22. The plants were appropriately spaced at 45 cm between rows and 15 cm between plants and

Table 1. List of iso-plasmic male sterile lines and restorer with their pedigree

Sl. No.	Male sterile lines	Pedigree
1	ICSA 11A$_4$ (M)	[(BTx624 × UChV2)B lines bulk]-5-1-1-1
2	ICSA 17A$_4$ (M)	[(BTx624 × 1807B)B lines bulk]-18-1-1
3	ICSA 26A$_4$ (M)	[(296B × BTx624)B lines bulk]-2-1-1-3
4	ICSA 88004A$_4$ (M)	[(([SC 108-3 × Swarna) × IS 9327]-6-2-2) × ((SC 108-3 × E35-1)-25-1)) × ([(BTx678 × UChV2)B lines bulk]-3-5-4-4)]-4-2-1-1
5	ICSA 88005A$_4$ (M)	[(([BTx624 × UChV2)B lines bulk]-5-1-1-1 × ([Btx623 × UChV2)B lines bulk]-10-1-4)) × DM 50] -1-1-1-1
6	M31-2A (M)	It is a natural mutant of M 35-1 (UAS, Raichur)
Sl. No.	**Restorer on *maldandi***	**Pedigree**
1	DSMR 8	Mutant line derived from BRJ 67

M- Maldandi cytoplasm

Table 2. Spikelet's fertility classes Based on seed set percentage

Category	Seed set per cent
Strong restoration	>90%
High restoration	80 to 90%
Moderate restoration	60 to 80%
Partial restoration	10 to 60%
Maintainer	0%

each entry was planted in two rows, each row being 3.0 meters length. The recommended package of practices and plant protection measures were under taken at appropriate time.

Before flowering, panicle from random five plants within each entry were covered to avoid outcrossing. These selfed panicles are used for recording seed set percentage for estimating fertility restoration.

2.2.1 Pollen fertility

The assessment of pollen fertility was carried out using 2 per cent acetocarmine stain. Anthers from five random panicles from each entry were collected and pollen grains were extracted from anthers onto glass slide. The 2% acetocarmine stain is added on the pollen grains and left for few minutes for proper staining. Using binocular microscope, the total number of fertile and sterile pollen grains were recorded in five microscopic fields within each glass slide. Pollen grains that were well stained and completely round were categorized as fertile whereas, those that were unstained or partially stained or shrivelled were considered sterile. This counting and assessment of fertility/sterility percentage was performed for each cross [23].

> Pollen fertility (%) = Number of stained pollen grains / Total number of pollen grains * 100

2.2.2 Seed set percentage:

To work out seed set percentage, the selfed panicle was divided into three parts and within each part five primaries were selected. The total number of spikelets and seeds were recorded using these selected primaries. The percentage of seed setting was determined using the formula [24].

> Seed set % = Total number of seeds / Total number of spikelet's * 100

Based on seed set percentage, the individuals were categorized into different fertility classes [24].

3. RESULTS AND DISCUSSION

The implementation of the cytoplasmic-genetic male sterility (CGMS) system paved the way for the commercial utilization of heterosis in sorghum. The CGMS system is governed by the interaction between cytoplasmic and nuclear genes. It was observed that the male fertility restoration in various CMS inducing sorghum cytoplasms is regulated by one or a few dominant genes that selectively interact with specific cytoplasmic genes [25,26].

3.1 Pollen Fertility and Seed Set

In this current investigation, the pollen fertility of the hybrids varied from 69.82 to 92.42 percent

(Table 2). Out of six hybrids, M31-2A (M) × DSMR 8 exhibited highest and ICSA 88004 A_4 (M) × DSMR 8 showed lowest pollen fertility of 92.42 and 69.82 per cent, respectively (Fig. 1). The remaining hybrids ICSA 26 A_4 (M) × DSMR 8, ICSA 17 A_4 (M) × DSMR 8, ICSA 88005 A_4 (M) × DSMR 8 and ICSA 11 A_4 (M) × DSMR 8 recorded 82.67, 78.99, 77.25 and 72.83 mean pollen fertility percentage, respectively.

For the seed set percentage, the mean value ranged from 61.19 (ICSA 88004 A_4 (M) × DSMR 8) to 85.17 per cent (M31-2A (M) × DSMR 8) (Fig. 2) (Table 3). Out of the six hybrids, two crosses exhibited high restoration (>80 %) and remaining four crosses showed moderate restoration (60-80%). The four hybrids *viz.,* ICSA 17 A_4 (M) × DSMR 8, ICSA 88005 A_4 (M) × DSMR 8, ICSA 88004 A_4 (M) × DSMR 8 and, ICSA 11 A_4 (M) × DSMR 8 recorded 72.40, 72.22, 67.48 and 61.19 mean seed set percentage, respectively.

The results for pollen fertility and seed set percentage in the F_1's showed differential behaviour of pollen parents in restoring fertility in different CMS lines of same cyto-sterile source (Table 2 and Table 3) (Fig. 3). However, the seed set percentage is considered a more reliable measure of male fertility restoration in CMS lines. Because lower pollen fertility can still lead to higher seed set percentage. This phenomenon could be attributed to the production of very large number of pollen grains, among which only one effective pollen grain will be sufficient for effective fertilization. Similarly, in the present study, all the crosses exhibited lower pollen fertility compared to corresponding seed set percentage. If the proposition mentioned above is true, then all the F_1's with partial pollen fertility should show normal seed set fertility. Contrary to this expectation, practical observations do not support this, suggesting that even partially fertile pollen grains may germinate on the stigma and blocks the stylar path, leading to the failure of fertilization by normal pollen grains. Further investigation is needed to explore this aspect [27].

Table 3. Pollen fertility percentage in iso-plasmic hybrids

Sl. No.	Hybrids	Number of stained pollen	Total number of pollen	Pollen fertility (%)
1	ICSA 11 A_4 (M) × DSMR 8	319.28	438.40	72.83
2	ICSA 17 A_4 (M) × DSMR 8	313.00	396.25	78.99
3	ICSA 26 A_4 (M) × DSMR 8	320.20	387.30	82.67
4	ICSA 88004 A_4 (M) × DSMR 8	288.10	412.65	69.82
5	ICSA 88005 A_4 (M) × DSMR 8	250.82	324.70	77.25
6	M31-2A (M) × DSMR 8	378.40	409.50	92.42
Mean				79.00
Standard Deviation				7.29

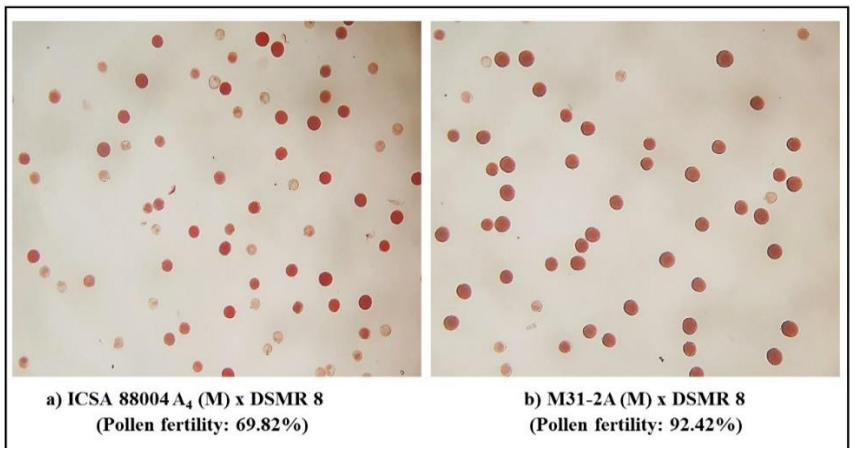

Fig. 1. Differential pollen fertility in hybrids generated with iso-plasmic male sterile lines

Table 4. Seed set percentage in iso-plasmic hybrids of sorghum

Sl. No.	Hybrids	Number of spikelets	Number of seeds	Seed set (%)	Fertility status
1	ICSA 11 A_4 (M) × DSMR 8	2310.00	1413.40	61.19	Moderate restoration
2	ICSA 17 A_4 (M) × DSMR 8	1143.60	822.60	72.40	Moderate restoration
3	ICSA 26 A_4 (M) × DSMR 8	1193.00	962.60	80.17	High restoration
4	ICSA 88004 A_4 (M) × DSMR 8	1181.00	797.20	67.48	Moderate restoration
5	ICSA 88005 A_4 (M) × DSMR 8	1012.20	727.60	72.22	Moderate restoration
6	M31-2A (M) × DSMR 8	750.60	638.60	85.17	High restoration
Mean				73.11	
Standard Deviation				7.86	

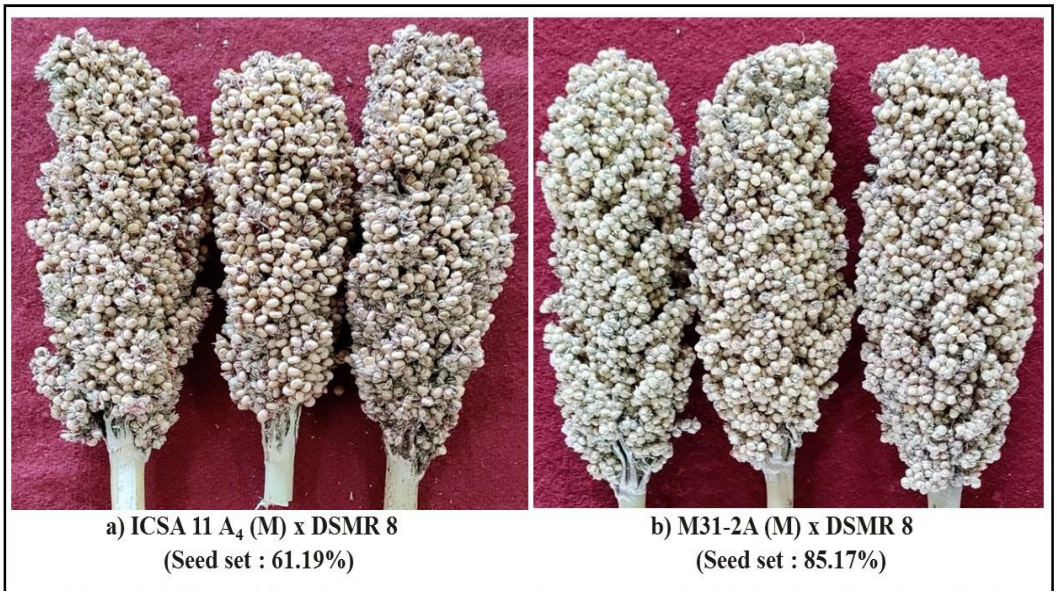

Fig. 2. Differential seed set percentage in hybrids generated with iso-plasmic male sterile lines

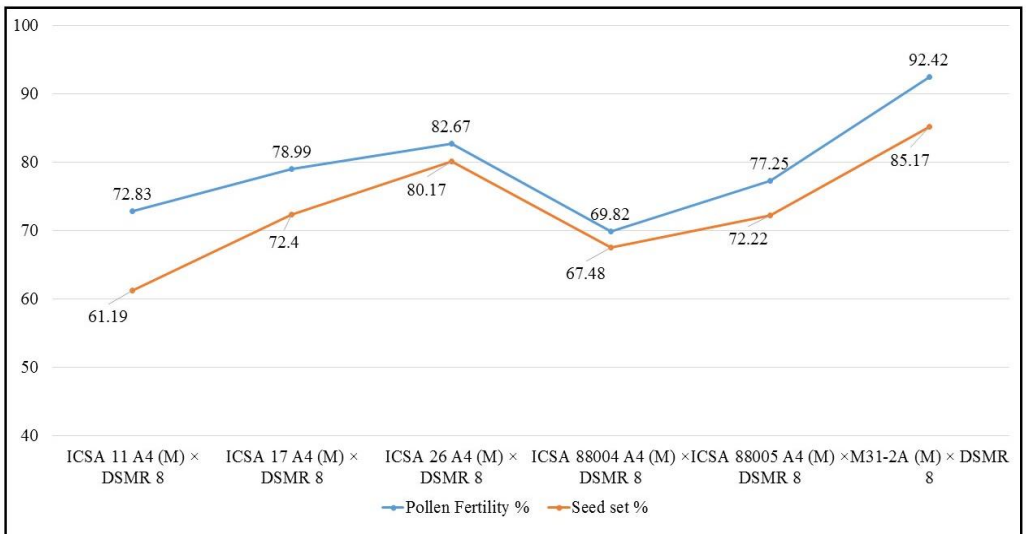

Fig. 3. Graph depicting the pollen fertility and seed percentage among sis iso-plasmic hybrids

The probable reasons for this differential expression of restorer gene could be due to distinct nuclear backgrounds of the CMS line. Similar variation in the effect of restorer gene with same cytosterile system was also reported [28-31].

The variation in fertility restoration expression is hypothesized to arise from an abundance of sterility nuclear genes in the female parent. These genes could potentially inhibit pollen fertility restoration in the hybrids. Consequently, a more efficient restorer line would likely possess an array of restorer genes along with additional minor fertility genes, which function in a complementary or additive manner to achieve complete fertility restoration [32].

Other possible reasons could be the distinct interaction between the cytoplasm and nuclear backgrounds of the female parent with the pollen parent [33], as well as the influence of modifier or minor genes present in the pollen parent could also leads to varying fertility restoration ability [34]. Furthermore, the expressivity of the restorer gene(s) can vary in different genetic backgrounds of the female parent [35]. The above reasons collectively provide potential explanations for the observed variation in fertility restoration.

4. CONCLUSION

The results of current study clearly reveal that although the CMS lines belongs to the same cyto-sterile source, the restorer lines show differential fertility restoration capacity in combination with different CMS lines. The differential reaction of CMS lines with the same restorer line reflects the profound influence of nuclear background of the female parent on the expression of fertility in the hybrid progenies. Further the same experiment need to be conducted in replicated trial to confirm the variations for pollen fertility and seed set percentage and these six hybrids should be forwarded to F_2 generation to understand the precise reasons responsible for differential expression of pollen fertility and seed set percentage.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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