

Differential expression of restorer gene on different nuclear background with *maldandi* cytoplasm in sorghum (*Sorghum bicolor* (L.) Moench)

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 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 [16,17 and 18], post-translation [19], and metabolic [20] levels which ultimately results in the production

of fertile F₁ 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* (A₄) 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 material:

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

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 <i>maldandi</i>	Pedigree
1	DSMR 8	Mutant line derived from BRJ 67

M- *Maldandi* cytoplasm

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 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].

$$\text{Pollen fertility (\%)} = \frac{\text{Number of stained pollen grains}}{\text{Total number of pollen grains}} \times 100$$

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].

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

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

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%

3. Results and Discussion

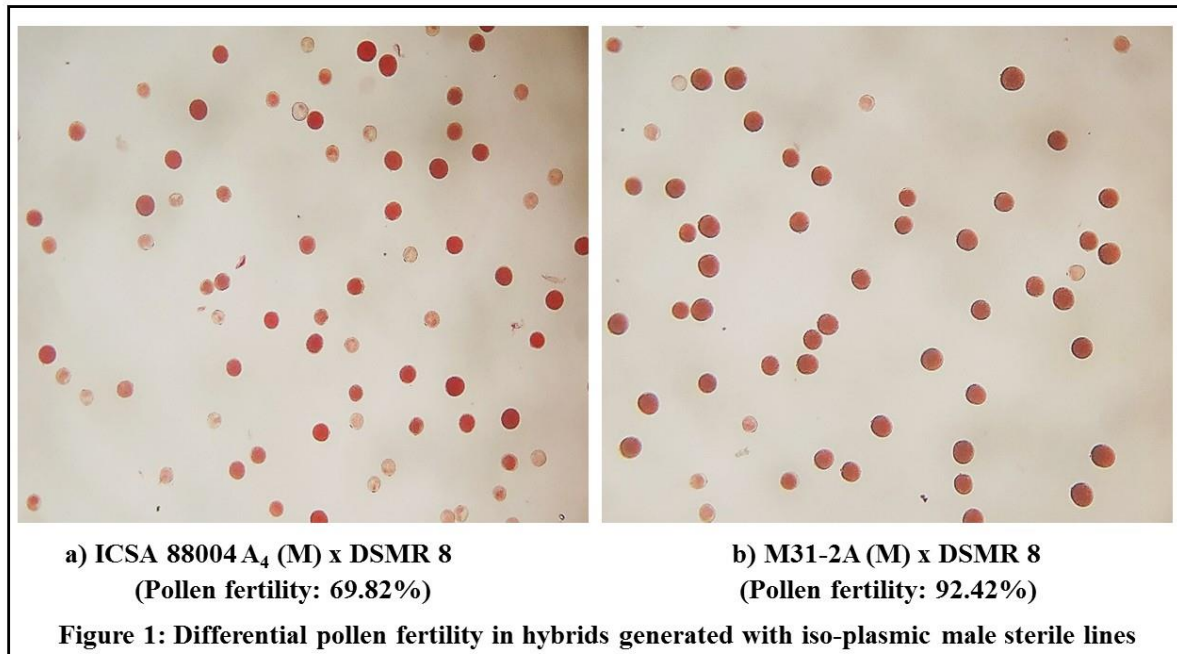
The implementation of the cytoplasmic-genetic male sterility (CGMS) system paved the way for the commercial utilization of heterosis in sorghum. The CGMS system is governed by the interaction between cytoplasmic and nuclear genes. It was observed that the male fertility restoration in various CMS inducing 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 A₄ (M) × DSMR 8 showed lowest pollen fertility of 92.42 and 69.82 per cent, respectively (Fig. 1). The remaining hybrids ICSA 26 A₄ (M) × DSMR 8, ICSA 17 A₄ (M) × DSMR 8, ICSA 88005 A₄ (M) × DSMR 8 and ICSA 11 A₄ (M) × DSMR 8 recorded 82.67, 78.99, 77.25 and 72.83 mean pollen fertility percentage, respectively.

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

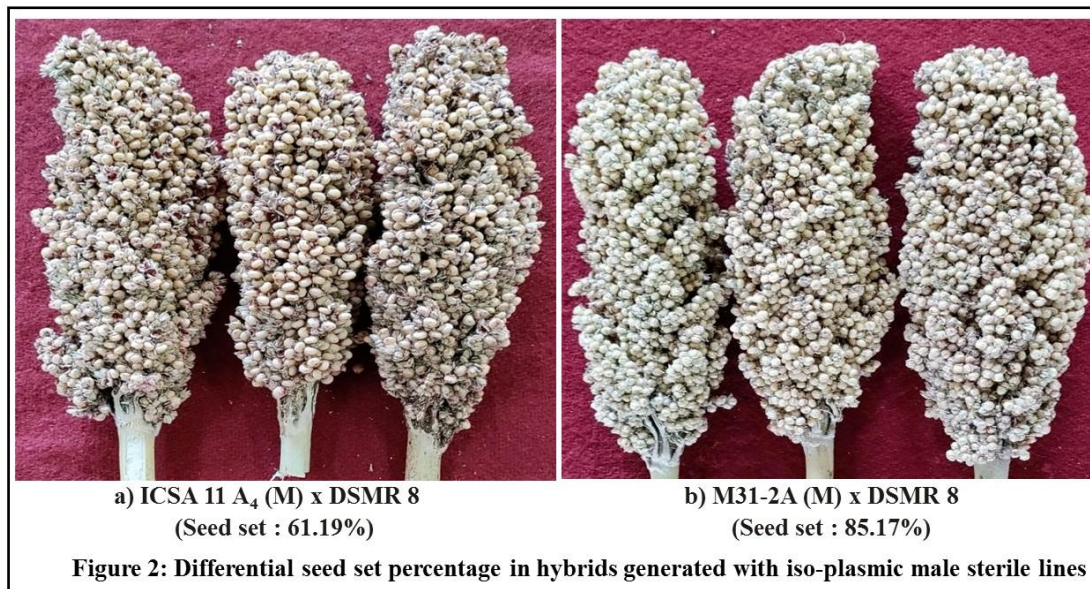


For the seed set percentage, the mean value ranged from 61.19 (ICSA 88004 A₄ (M) × DSMR 8) to 85.17 per cent (M31-2A (M) × DSMR 8) (Fig. 2) (Table 3). Out of the six hybrids, two crosses exhibited high restoration (>80 %) and remaining four crosses showed moderate

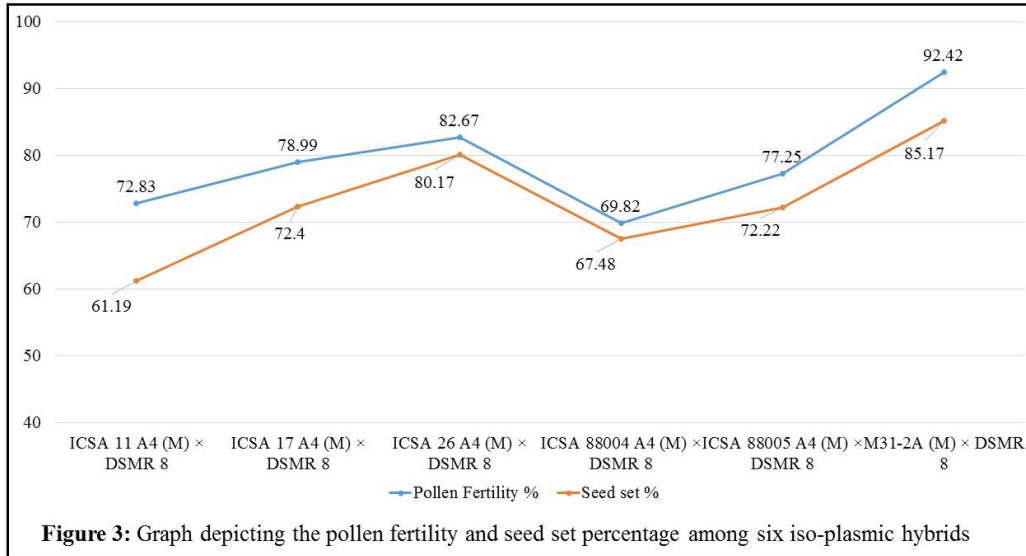
restoration (60-80%). The four hybrids *viz.*, ICSA 17 A₄ (M) × DSMR 8, ICSA 88005 A₄ (M) × DSMR 8, ICSA 88004 A₄ (M) × DSMR 8 and, ICSA 11 A₄ (M) × DSMR 8 recorded 72.40, 72.22, 67.48 and 61.19 mean seed set percentage, respectively.

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	



The results for pollen fertility and seed set percentage in the F₁'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 F₁'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].



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 cyto-sterile system was also reported [28,29,30 and 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 F₂ generation to understand the precise reasons responsible for differential expression of pollen fertility and seed set percentage.

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Author contribution

Research conceptualization (BDB); Experiment design (BDB, NGH); Field/lab work and data collection (PKN); Data analysis and interpretation (PKN, BDB, NGH); Manuscript drafting (PKN); Manuscript revision (BDB, NGH, PSK, RS). All authors contributed and approved the final manuscript.

References

- [1] Khalid W, Ali A, Arshad MS, Afzal F, Akram R, Siddeeg A et al. Nutrients and bioactive compounds of *Sorghum bicolor* L. used to prepare functional foods: a review on the efficacy against different chronic disorders. *Int J Food Prop.* 2022;25(1): 1045-1062. DOI: <http://dx.doi.org/10.1080/10942912.2022.2071293>.
- [2] Rao BD. Sorghum value chain for food and fodder security. In *Breeding sorghum for diverse end uses* (pp. 409-419). Woodhead Publishing;2019.
- [3] Hanson MR, Bentolila S. Interactions of mitochondrial and nuclear genes that affect male gametophyte development. *Plant Cell.* 2004;16: 154-169. DOI: <http://dx.doi.org/10.1105/tpc.015966>.
- [4] Duan LY, Wu T, Li X, Xie JK, Hu BL. Progress on Cytoplasmic Male Sterility and Fertility Restoration Genes in Rice. *Crops.* 2022;38: 20-30.
- [5] Schnable PS, Wise RP. The molecular basis of cytoplasmic male sterility and fertility restoration. *Trends Plant Sci.* 1998;3(5): 175-180. DOI: [http://dx.doi.org/10.1016/S1360-1385\(98\)01235-7](http://dx.doi.org/10.1016/S1360-1385(98)01235-7).
- [6] De Souza A, Wang JZ, Dehesh K. Retrograde signals: integrators of interorganellar communication and orchestrators of plant development. *Annu Rev Plant Biol.* 2017;68: 85-108. DOI: <http://dx.doi.org/10.1146/annurev-arplant-042916-041007>
- [7] Luo D, Xu H, Liu Z, Guo J, Li H, Chen L et al. A detrimental mitochondrial-nuclear interaction causes cytoplasmic male sterility in rice. *Nat Genet.* 2013;45(5): 573-577. DOI: <http://dx.doi.org/10.1038/ng.2570>.
- [8] Xiao S, Zang J, Pei Y, Liu J, Liu J, Song W et al. Activation of mitochondrial orf355 gene expression by a nuclear-encoded DREB transcription factor causes cytoplasmic male sterility in maize. *Mol Plant.* 2020;13(9): 1270-1283. DOI: <http://dx.doi.org/10.1016/j.molp.2020.07.002>
- [9] Chen L, Liu YG. Male sterility and fertility restoration in crops. *Annu Rev Plant Biol.* 2014;65: 579-606. DOI: <http://dx.doi.org/10.1146/annurev-arplant-050213-040119>
- [10] Dewey RE, Timothy DH, Levings CS. A mitochondrial protein associated with cytoplasmic male sterility in the T cytoplasm of maize. *PNAS.* 1987;84(15): 5374-5378. DOI: <http://dx.doi.org/10.1073/pnas.84.15.5374>.

- [11] Pring DR, Schertz KF. Cytoplasmic male sterility and organellar DNAs of sorghum. In C.S Levings, III and I. K. Vasil (Eds.), *Mol Biol Plant Mitochondria*. 1995;3: 461-495.
- [12] Colombo N, Galmarini CR. The use of genetic, manual and chemical methods to control pollination in vegetable hybrid seed production: a review. *Plant Breed*. 2017;136(3): 287-299. DOI: <http://dx.doi.org/10.1111/pbr.12473>
- [13] Mackenzie SA and Chase CD. Fertility Restoration Is Associated with Loss of a Portion of the Mitochondrial Genome in Cytoplasmic Male-Sterile Common Bean. *Plant Cell*. 1990;2(9): 905-912. DOI: <http://dx.doi.org/10.2307/3869326>.
- [14] Ning L, Wang H, Li D, Li Y, Chen K, Chao H et al. Genome-wide identification of the restorer-of-fertility-like (RFL) gene family in *Brassica napus* and expression analysis in Shaan2A cytoplasmic male sterility. *BMC Genom*. 2020;21(1): 1-14. DOI: <http://dx.doi.org/10.1186/s12864-020-07163-z>.
- [15] Hu J, Wang K, Huang W, Liu G, Gao Y, Wang J et al. The rice pentatricopeptide repeat protein *RF5* restores fertility in Hong-Lian cytoplasmic male-sterile lines via a complex with the glycine-rich protein *GRP162*. *Plant Cell*. 2012;24(1): 109-122. DOI: <http://dx.doi.org/10.1105/tpc.111.093211>.
- [16] Koizuka N, Imai R, Iwabuchi M, Sakai T, Imamura J. Genetic analysis of fertility restoration and accumulation of ORF125 mitochondrial protein in the kosenia radish (*Raphanus sativus* cv. Kosenia) and a *Brassica napus* restorer line. *Theor Appl Genet*. 2000;100: 949-955. DOI: <http://dx.doi.org/10.1007/s001220051375>.
- [17] Koizuka N, Imai R, Fujimoto H, Hayakawa T, Kimura Y, Kohno-Murase J et al. Genetic characterization of a pentatricopeptide repeat protein gene, orf687, that restores fertility in the cytoplasmic male-sterile Kosenia radish. *Plant J*. 2003;34(4): 407-415. DOI: <http://dx.doi.org/10.1046/j.1365-313X.2003.01735.x>.
- [18] Wang C, Lezhneva L, Arnal N, Quadrado M, Mireau H. The radish Ogura fertility restorer impedes translation elongation along its cognate CMS-causing mRNA. *PNAS*. 2021;118(35): 1-9. DOI: <http://dx.doi.org/10.1073/pnas.2105274118>.
- [19] Kitazaki K, Arakawa T, Matsunaga M, Yui-Kurino R, Matsuhira H, Mikami T et al. Post-translational mechanisms are associated with fertility restoration of cytoplasmic male sterility in sugar beet (*Beta vulgaris*). *Plant J*. 2015;83(2): 290-299. DOI: <http://dx.doi.org/10.1111/tpj.12888>.
- [20] Liu F, Cui X, Horner HT, Weiner H, Schnable PS. Mitochondrial aldehyde dehydrogenase activity is required for male fertility in maize. *Plant Cell*. 2001;13(5): 1063-1078. DOI: <http://dx.doi.org/10.2307/3871364>.
- [21] Ramalingam J, Nadarajan N, Rangaswamy P, Vanniarajan C. Genetic analysis of fertility restoration in hybrid rice (*Oryza sativa* L.). *Ann Agric Res*. 1992;13: 221-223.
- [22] Kumar AA, Reddy BVS, Kaul SL. Alternate cytoplasmic male sterility system in sorghum and their utilization. In: Reddy B V S, Ramesh S, Kumar A A and Gowda C L L (Eds.) *Sorghum Improvement in the New Millennium. Patancheru. 502 324, Telangana, India:*

International Crops Research Institute for the Semi-Arid Tropics. 340 pp. ISBN 978-92-9066-512-0;2008.

- [23] Alexander MP. Differential staining of aborted and non aborted pollen. *Stain Technol.* 1969;44(3): 117-122.
- [24] Biradar BD. Genetic studies involving diverse source of cytoplasm-genetic male sterility in sorghum [*Sorghum bicolor* [L.] Moench]. *Ph. D. Thesis*, University of Agricultural Sciences, Dharwad, India;1995.
- [25] Schertz KF, Pring DR. Cytoplasmic sterility systems in sorghum. *Sorghum in the Eighties*, 1982;1: 373-383.
- [26] Tripathi DP, Rana BS, Rao NGP. (1985). Genetics of fertility restoration in sorghum. *Indian J Genet Plant Breed.* 1985;45(2): 292-301.
- [27] Hariprasanna K, Zaman FU, Singh AK. Nucleo-cytoplasmic interactions for fertility restoration in wild aborted (wa) CMS lines of rice (*Oryza sativa* L.). *Crop Res.* 2006;31(1): 128-134.
- [28] Govindaraj K, Virmani SS. Genetics of fertility restoration of 'WA' type cytoplasmic male sterility in rice. *Crop Sci.* 1988;28(5): 787-792.
- [29] Salgotra RK, Katoch PC, Kaushik RP. Identification of restorers and maintainers for cytoplasmic genic male sterile lines of rice. *Oryza.* 2002;39: 55–57.
- [30] Bhadru D, Reddy DL, Ramehsa MS. Differential reaction of selected restorers/maintainers with WA based CMS lines and genetics of fertility restoration in hybrid rice (*Oryza sativa* L.). *Agric Sci Digest.* 2015;35(2): 116-120. DOI: <http://dx.doi.org/10.5958/0976-0547.2015.00020.8>.
- [31] Naik RK, Babu PR, Babu JDP, Rani YA, Rao VS. Differential reaction of selected restorers/maintainers with WA based CMS lines in hybrid rice (*Oryza sativa* L.). *J. Res. ANGRAU*, 2018;46(3): 9-17.
- [32] Virmani SS, Govinda RK, Casal CL, Dalmacio RD, Aurin PA. Current knowledge of and outlook on cytoplasmic-genetic male sterility and fertility restoration in rice. In *Rice Genetics*. 1986;633-647. DOI: http://dx.doi.org/10.1142/9789812814265_0054.
- [33] Leenakumari S, Mahadevappa M. Nucleo-cytoplasmic interaction for fertility restoration in cytoplasmic male sterile lines of rice. *Oryza*, 1998;35: 124-126.
- [34] Ganesan KN, Rangaswamy M, Thiyagarajan K. Effect of minor genes in restoration of fertility in CMS lines of rice. *Int Rice Res Notes.* 1998;23(1): 1-1.
- [35] Leenakumari S, Mahadevappa M, Kulkarni RS. Fertility restoration studies in four WA CMS lines of rice. *Int Rice Res Notes.* 1998;23(1): 1-1.

