

Genetics of Fertility Restoration in A₁ Cytoplasmic Genetic Male Sterility (CGMS) Systems in Sorghum (*Sorghum bicolor* L. Moench)

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Abstract

The cause of Cytoplasmic Genetic Male Sterility (CGMS) is specific nuclear and mitochondrial interactions. Almost all commercial sorghum hybrids were developed using the A1 cytoplasmic genetic male sterility system. Understanding the inheritance of fertility restoration in sorghum for A1 cytoplasm, for example, can improve the selection efficiency of restorer lines for increased seed production. In a cross of male sterile line 296A with A1 cytoplasm and restorer lines comprised of a set of Recombinant Inbred Lines (RILs), the inheritance pattern of fertility restoration of sorghum was studied. The F_1 hybrid was completely fertile, revealing the dominant nature of fertility restoration, which is controlled by one or two major genes with modifiers. In this study, the genetics of fertility restoration of the A1 cytoplasmic nuclear male sterility system (CGMS) in sorghum were investigated in segregating F_2 and BC_1 populations of A1 cytoplasm crosses. Fertility restoration was governed by a monogenic inheritance (3F:1S) mechanism represented by a single dominant gene responsible for fertility restoration in all of the crosses studied.

1. Introduction

Sorghum originated in Africa and evolved into an important cereal crop. It now feeds over 500 million people in 98 countries, with 42 million hectares of cultivated land and 62 million tonnes of yields per year. The global sorghum crop area is 39.93 million hectares, with a total production of 59.35 million tonnes and a productivity of 6.7 metric tonnes per hectare (FAOSTAT). The interaction of sterility-inducing factors in the cytoplasm with genetic factors in the nucleus causes cytoplasmic-genetic male sterility (CGMS). This system focuses on three lines of breeding: A-, B-, and R-lines. Because most CMS lines have irregular anther or pollen formation, the A-line is a male sterile. When the B- and A-lines were crossed, they produced genetically male sterile offspring. The main difference between A-line and B-line deals with flowering. The third line is called the restorer line (R-line). The restorer line's purpose is to serve as a pollinator variety for pollinating the CMS line in order to produce F1 hybrids (Yuan et al. 2003). It is essential for the restorer line to have strong restoring ability. To ensure successful pollen transfer from R-line to A-line, the restorer line should have venerable combining ability and agronomic characteristics, as well as a well developed flowering system. Stephens and Holland (1954) discovered A1 cytoplasmic genic male sterility (CGMS) in sorghum and exploited it for hybrid production worldwide. The genetic makeup of the cytoplasm and nuclei determines the inheritance of male sterility/fertility. In some cases, single genes control male fertility restoration, but it is polygenic when the same nuclear genotype interacts with different cytoplasm. Intra and inter-allelic interactions and complementation influence fertility restoration. The thorough understanding of the genetics of fertility restoration is useful in planning a sound breeding strategy for the development of superior restorers in a hybrid breeding program. It may also help in the efficient transfer of restorer genes into other agronomical desirable genotypes. Cytoplasmic male sterility which

causes the production of non-functional pollen and inherited maternally is important in commercial hybrid seed production and breeding program. In last few decades, productivity of field, vegetables and fruit crops was increased due to this hybrid breeding technique. Efficiency of exploitation of heterosis at commercial level increased due to availability of more number of cytoplasmic genetic male sterile sources for fertility restoration. Dominant fertility restoring nuclear genes are transmitted from the male parent, which allow seed set on the hybrid plants. However, the expression of fertility restoration may vary from 0 % to 100 % fertility restoration of CGMS-based hybrids is an integral part of breeding hybrids and the development of new hybrid parents with desirable agronomic and market preferred traits on regular intervals is essential for the sustainability of hybrid technology programs. The presence of greater genetic diversity among fertility restorers enhances the probability of breeding widely adapted high yielding hybrids (Dandin et al. 2014; Praveen et al. 2015). The information about the number of genes controlling fertility restoration in the nucleus suppresses the male-sterile phenotype and allows commercial exploitation of CMS system for the production of hybrid seeds. Therefore, the present investigation was undertaken to assess the genetics of fertility restoration system in sorghum using F_2 and BC_1F_1 generations in three sorghum hybrids carrying A_1 cytoplasm.

2. Materials and Methods

Cytoplasmic genetic male sterile line (296A) crossed with 238 RILs (Recombinant Inbred Lines) individuals (296B x IS 18551) to produced F_1 hybrids, evaluated in rainy and post-rainy season 2016 for seed setting percentage and pollen fertility/viability score. From these multi-season evaluation program, results revealed that three F_1 's viz; 296A x ICSL 43119, 296A x ICSL 43123 & 296A x ICSL 43126 were selected to study genetics of fertility restoration on the basis of high seed set percent (> 90 %) and high pollen fertility/viability score (8-9 score). All these selected F_1 plants were selfed to produce F_2 seeds and

simultaneously crossed all the F₁ plants to their male sterile line (296A) to produced BC₁ seeds. The F₂'s and BC₁s along with their parents were raised at International Crops Research Institute for Semi-Arid Tropics, Patancheru, India during late post-rainy season 2017-18. Prior to flowering, heads from each line were covered with paper bags to exclude foreign pollen. At 40 days after the crop flowered, the bags were removed and the percent seed set on each head was visually rated. On the basis of seed setting percentage the F₂ and BC₁ classified into fertile, partial fertile and sterile (Prasad and Biradar, 2018). The F₂ and BC₁ populations were tested for segregation ratios to determine the number of genes involved in the fertility restoration of cytoplasmic genetic male sterility (CGMS) systems. The goodness of fit to the expected ratios in F₂ and BC₁F₁ generations were tested using chi-square test (Meshram and Patil, 2018; Praveen et al., 2015, Arunkumar et al., 2004; Dandin et al., 2014).

3. Statistical Analysis

All the data analysis was carried out in R software version 3.6.3 (R Core Team, 2019). Chi square (χ^2) method with Yates correction factor (Steel and Torrie, 1980) was applied on the observed data to test the goodness of fit of different genetic ratios. The calculated χ^2 values were compared with the tabulated χ^2 values with (n-1) degrees of freedom at 5 % and 1 % probability level. The null hypothesis was rejected if the calculated χ^2 value exceeded the corresponding tabulated χ^2 value. Exact probability value at (n-1) degrees of freedom for the best fit hypothetical ratio was calculated in the Excel spreadsheet using the statistical function 'CHIDIST'.

$$\chi^2 \text{ cal} = \sum [(O-E) - 0.5]^2 / E$$

Where,

O = observed number of plants

E = expected number of plants

Σ = summation over all classes

n = number of independent classes in the hypothetical distribution

4. Results

The inheritance of A₁ CMS system was investigated on F₂ and BC₁ in a total of 3 (A x R) crosses. All the F₂s and BC₁F₁s were evaluated at ICRISAT-Patancheru for seed setting data. In crosses where seed setting data was recorded, genetic ratios were worked out by distribution of plants in their respective groups viz; seed setting percentage between 0-10 % classified as sterile, seed setting percentage between 11-60 % were grouped as partial sterile while seed setting percentage between 61-100 % were considered as fertile (Prasad and Biradar, 2018). The results of inheritance of A₁ CMS system with respect to each of the fertility restorer parents have been presented below.

In the present study, In cross 296A x ICSL 43119, 610 F₂ plants segregated in 458 fertile (F) and 152 sterile (S) plants and had a good fit ($\chi^2 = 0.0$; P = 0.963) to the ratio of 3F:1S which revealed single dominant gene responsible for fertility restoration. 590 BC₁F₁ plants of the same distributed in 299 fertile (F) and 291 sterile (S) and show segregation of 1F:1S ($\chi^2 = 0.11$; P = 0.742). In cross 296A x ICSL 43123, 595 F₂ plants segregated in 430 fertile (F) and 165 sterile (S) and fit to the hypothesized 3F:1S ratio ($\chi^2 = 2.37$, P = 0.124). In the same way, 585 BC₁F₁ plants divided in 301 fertile (F) and 284 sterile (S) show segregation according to ratio of 1F:1S as indicated by χ^2 value of 0.49 (P = 0.482). In cross 296A x ICSL 43126, 495 F₂ plants segregated in 375 fertile (F) and 120 sterile (S) had a good fit ($\chi^2 = 0.15$; P = 0.697) to 3F:1S while, 400 BC₁F₁ plants segregated in 208 fertile (F) and 192 sterile (S) was in good agreement with the expected ratio of 1F:1S as evident from χ^2 value of 0.64 (P = 0.424) (Table 1).

5. Discussion

Mechanisms by which male fertility restoration occurs are probably as diverse as mechanisms by which mitochondrial mutations cause CMS. Although restorer alleles are known to affect all the well-characterized CMS-associated genes, mechanism of action has not been determined definitively for any restorer allele. The knowledge of fertility restoration genetics is utmost important for the transfer of restorer genes from one genotype to another (Sawargaonkar et al. 2012). In certain cases, the environment also plays an important role in the expression of pollen fertility. The presence of homozygous recessive alleles at one locus produces partial fertility, whereas the presence of fertility restoring alleles at the other locus produces male sterility (Saroj et al., 2015).

In the present study, The A₁ cytoplasm based Recombinant Inbred Line (RIL) hybrids were evaluated multi season for seed setting percentage and pollen fertility score. Seed setting percentage and pollen fertility score recorded in rainy season on Recombinant Inbred Line (RIL) population along with pollen fertility score recorded in post rainy season had a good fit to the monogenic expected ratio of 3:1 (Campa et al. 2014). 3 F₂s and 3 BC₁F₁s developed from single crosses *viz*; 296A x ICSL 43119, 296A x ICSL 43123 and 296A x ICSL 43126 evaluated to study genetics of fertility restoration. Monogenic ratio (3F:1S) showed non-significant χ^2 values concluding that observed and expected values are with very negligible and/or no differences (Ali et al., 2011; Reddy et al., 2010; Rongbai et al., 2005; Sawargaonkar et al., 2012; Sreedhar et al., 2011; Murty and Gangadhar, 1990). The results are discussed below.

In six segregating populations (3 F₂s and 3 BC₁F₁s) developed from three crosses, a monogenic F₂ ratio of 3F:1S and the corresponding BC₁ ratio of 1F:1S was observed that results from single dominant gene involved in fertility restoration. Consider 'A' gene involved in fertility restoration of the A₁ CMS system. The monogenic F₂ ratio of 3F:1S and

the corresponding BC₁F₁ ratio of 1F:1S is possible when the genotype of female parent is 'aa' and of restorer parents is 'AA' indicating that single dominant gene involved in fertility restoration. The F₁ of these parents will be fertile and heterozygous for single locus (Aa). A plant in the F₂ will be fertile if it possesses dominant allele of the basic gene/s. All other plants will be sterile. In backcross, the F₁ plant (Aa) crossed with female parent (aa). From this, half plants containing dominant allele of the basic gene will be fertile and others will be sterile. Monogenic modes of inheritance have been reported in sorghum for the A₁ (*nilo*) CMS system (Maunder and Pickett, 1959; Murty and Gangadhar, 1990; Arunkumar et al., 2004; Praveen et al., 2015), for A₂ (Praveen et al., 2015), for maldandi cytoplasm (Dandin et al., 2014) and for 9E and A₄ CMS systems (Elkonin et al., 1998); in pearl millet for A₄ CMS system (Gupta et al. 2012) and in A₅ (Gupta et al. 2018) in rice for CMS-BT (Komori et al., 2003), CMS-HL (Huang et al., 2000), in sunflower CMS-PET1 (Chandra et al., 2010; Sujatha et al., 2011) and for CMS-ANL2, CMS-PEF1 and CMS-PET2 (Chandra et al., 2010; Sujatha et al., 2011), in rice (Ahmadikhah et al., 2007; Sreedhar et al., 2011), in Brassica (Ahmad et al., 2013; Bisht et al., 2015), in pigeon pea (Sawargaonkar et al., 2012).

6. Conclusion

It is necessary to keep upgrading the hybrid technology so that high yielding hybrids can be developed. The knowledge on the genetics of fertility restoration helps in designing a plan for breeding elite hybrid parents. The genetics of fertility restoration of the A1 cytoplasmic male sterility system was governed by a single dominant gene in this study. Crosses among diverse restorer lines are required for CMS based hybrid breeding so that genotypes with high seed setting percentage can be selected.

REFERENCES

- Ahmad, R., Farhatullah., Khan, R. S., Quiros, C. F. 2013. Inheritance of fertility restorer gene for cytoplasmic male sterility in *B. Napus* and identification of closely linked molecular markers to it. *Euphytica* 194, 351-360.
- Ahmadikhah, A., Karlov, G. I., Nematzadeh, G., Bezdi, K. G. 2007. Inheritance of the fertility restoration and genotyping of the rice lines at the restoring fertility (*Rf*) loci using molecular markers. *Int. J. Plant. Protect.* 1(1), 13-21.
- Ali, A., Vinod., Tomar, S. M. S., Chand, S. 2011. Genetics of fertility restoration and test for allelism of restorer genes in wheat (*Triticum aestivum* L.). *Indian J. Genet.* 71(3), 223-230.
- Arunkumar, B., Biradar, B. D., Salimath, P. M. 2004. Inheritance of fertility restoration on milo and maldandi sources of male sterility in *rabi* sorghum [*Sorghum bicolor* (L.) Moench]. *Indian J. Genet.* 64(4), 325-326.
- Bhati, P. K., Singh, S. K., Singh, R., Kumar, V., Sharma, A. 2015. Genetics of fertility restoration in 'WA' cytoplasmic male sterility in rice (*Oryza sativa* L.). *Res. Environ. Life Sci.* 8(4), 611-612.
- Campa, A., Rodriguez-Suarez, C., Giraldez, R., Ferreira, J. J. 2014. Genetic analysis of the response to eleven *Colletotrichum lindemuthianum* races in a RIL population of common bean (*Phaseolus vulgaris* L.). *BMC Plant Biol.* 14:115. doi:10.1186/1471-2229-14-115.
- Chandra, B. S., Kumar, S.S., Rangnatha, A. R. G., Dudhe, M. Y. 2010. Inheritance of fertility restoration for different CMS sources in sunflower (*Helianthus annuus* L.). *SABRAO J. Breed. Genet.* 42(1), 46-50.
- Dandin, R., Biradar, B. D., Pattanshetti, S. K. 2014. Inheritance pattern of fertility restoration on maldandi cytoplasm in *rabi* sorghum [*Sorghum bicolor* (L.) Moench]. *Karnataka J. Agric. Sci.* 27(4), 522-523.
- Elkonin, L. A., Kozemyakin, V. V., Ishin, A. G. 1998. Nuclear cytoplasmic interactions in restoration of male fertility in '9E' and A₄ CMS-inducing cytoplasm of sorghum. *Theor. Appl. Genet.* 97, 626-632.
- Gupta, S. K., Rai, K. N., Govindaraj, M., Rao, A. S. 2012. Genetics of fertility restoration of the A₄ cytoplasmic nuclear male sterility system in pearl millet. *Czech. J. Genet. Plant Breed.* 48(2), 87-92.
- Gupta, S. K., Yadav, D. V., Govindaraj, M., Boratkar, M., Kulkarni, V. N. Rai, K. N. 2018. Inheritance of fertility restoration of A₅ cytoplasmic-nuclear male sterility system in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Indian J. Genet.* 78(2), 228-232.
- Hasan, M. J., Kulsum, U., Elahi, N. E., Shamsuddin, A. K. M., Raahman, M. M. 2015. Inheritance of fertility restoration involving ID type cytoplasmic male sterility system in rice (*Oryza sativa* L.) using ten different restorer lines. *SAARC. J. Agri.* 13(1), 207-215.

- Hasnunnahar, M., Khan, M. M. R., Isshiki, S. 2012. Inheritance analysis of fertility restoration genes (*Rf*) in a male sterile system of eggplant using cytoplasm of *Solanum grandiflorum*. *AJCS* 6(3), 475-479.
- Hossain, M. S., Singh, A. K., Zaman, F. U. 2010. Genetics of fertility restoration of 'WA' based cytoplasmic male sterility in rice (*Oryza sativa* L.) using indica/japonica derivative restorers. *Sci. Asia* 36, 94-99.
- Huang, Q. Y., He, Y. Q., Jing, R. C., Zhu, R. S., Zhu, Y. G. 2000. Mapping of the nuclear fertility restorer gene for HL cytoplasmic male sterility in rice using microsatellite markers. *Chinese Sci. Bul.* 45, 430-432.
- Komori, T., Yamamoto, T., Takemori, N., Kashiara, M., Matsushima, H., Nitta, N. 2003. Fine genetic mapping of the nuclear gene, *Rf₁*, that restores the BT-type cytoplasmic male sterility in rice (*Oryza sativa* L.) by PCR based markers. *Euphytica* 129, 241-247.
- Maunder, A. B., Pickett, R. C. 1959. The genetic inheritance of cytoplasmic genetic male sterility in grain sorghum. *Agron J.* 51, 47-49.
- Meshram, M. P., Patil, A. N. 2018. Genetics of fertility restoration in A₂ cytoplasm based hybrids of pigeon pea [*Cajanus cajan* (L.) Millsp.]. *Int. J. Curr. Microbiol. App. Sci.* 6, 565-571.
- Murty, U. R., and Gangadhar, G. 1990. Milo and non-milo sources of cytoplasm in *Sorghum bicolor* (L.) Moench. III. Genetics of fertility restoration. *Cereal Res. Commun.* 18(1-2), 111-116.
- Prasad, B.H.V., Biradar, B. D. 2018. Molecular characterization of fertility restorer genes for milo and maldandi cytoplasm from mini-core collection of *rabi* sorghum. *Int. J. Curr. Microbiol. App. Sci.* 7, 535-545.
- Praveen, M., Uttam, A., N, Suneetha., Av, Umakanth, Patil, J. V. and R, Madhusudhana. 2015. Inheritance and molecular mapping of *Rf₆* locus with pollen fertility restoration ability on A₁ and A₂ cytoplasm in sorghum. *Plant Sci.* 238, 73-80.
- Reddy, P. S., Rao, D. M., Reddy, B.V. S., and Kumar, A. A. 2010. Inheritance of male fertility restoration in A₁, A₂, A₃ and A₄ (M) cytoplasmic male sterility systems of sorghum [*Sorghum bicolor* (L.) Moench]. *Indian J. Genet.* 70(3), 240-246.
- Rongbai, Li., Pandey, M. P., and Sharma, P. 2005. Inheritance of thermo-sensitive genic male sterility in rice. *Curr. Sci.* 88(11), 1809-1815.
- Sarkar, C. K. G., Zaman, F. U., and Singh, A.K. 2002. Genetics of fertility restoration of 'WA' based cytoplasmic male sterility in rice (*Oryza sativa* L.) using basmati restorer lines. *Indian. J. Genet.* 62(4), 305-308.
- Saroj, S. K., Singh, M. N., Bhanu, A. N., Pathak, N., Dhurai, S. Y. and Yashpal. 2017. Genetics of fertility restoration and agronomic performance of CMS based hybrids in pigeonpea. *Int J. Agric. Env. Biot.* 10(1), 17-24.

Sawargaonkar, S. L., Madrap, I. A., and Saxena, K. B. 2012. Study of inheritance of fertility restoration in pigeon pea lines derived from *Cajanus cajanifolius*. Plant Breeding 131, 312-314.

Steel, RGD., Torrie, J. H., 1980. Principles and Procedures of Statistics.

Stephens, J. C. and Holland, R. F. 1954. Cytoplasmic male sterility for hybrid sorghum seed production. Agronomy J. **46**: 20-23.

Sujatha, M., Reddy, A. V., and Shankar, A. S. 2011. Genetics of fertility restoration in sunflower (*Helianthus annuus* L.). Current Biotica. 5(1), 56-63.

Waghmode, B. D., and Mehta, H. D., 2011. Genetics of fertility restoration of diverse cytoplasmic sources in rice (*Oryza sativa* L.). Indian J. Genet. 71(1), 1-8.

Yadav, D., Gupta, S. K., Rajpurohit, P. S., and Behl, R. K. 2010. Inheritance of A₁ system of cytoplasmic nuclear male sterility in pearl millet [*Pennisetum glaucum* (L.) R. Br.], Cereal Res. Commun. 38, 285-293.

Yuan, L. P., Wu, X. J., Liao, F. M., Ma, G. H., Xu, Q. S. 2003. Hybrid Rice Technology. Beijing: China Agriculture Press; pp. 9-21.

Table 1: Segregation ratios for fertile and sterile plants in F₂ and backcross population derived from crosses based on A₁ cytoplasm based A-lines in sorghum. (Monogenic Interaction)

SN	Cross	Generations	No of Plants			Expected ratio (F:S)	χ^2 value	Probability value (P)
			Total	Fertile	Sterile			
1	296A x ICSL 43119	P₁	10	0	10			
		P₂	9	9	0			
		F₂	610	458	152	3:1	0.00	0.963 ns
		BC₁F₁	590	299	291	1:1	0.11	0.742 ns
2	296A x ICSL 43123	P₁	10	0	10			
		P₂	9	9	0			
		F₂	595	430	165	3:1	2.37	0.124 ns
		BC₁F₁	585	301	284	1:1	0.49	0.482 ns
3	296A x ICSL 43126	P₁	10	10	0			
		P₂	11	0	11			
		F₂	495	375	120	3:1	0.15	0.697 ns
		BC₁F₁	400	208	192	1:1	0.64	0.424 ns