



Research Article

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 F₁ 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 F₂ and BC₁ 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.

Keywords A₁ cytoplasm, CGMS, fertility restoration, genetics, hybrid sorghum

Introduction

Sorghum originated in Africa and evolved into an important cereal crop. 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 male sterile. When the B- and A-lines were crossed, they produced genetically male sterile offspring. The mean difference between A-line and B-line deals with fertility. 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 to produce F₁ hybrids [1]. It is essential for the restorer line to have a 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 [2] discovered A₁ cytoplasmic genic male sterility (CGMS) in sorghum and exploited it for hybrid production worldwide. The genetic makeup of the cytoplasm and

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nuclei determine 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. A 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 is inherited maternally is important in commercial hybrid seed production and breeding program. In the last few decades, the productivity of fields, vegetables, and fruit crops was increased due to this hybrid breeding technique. The efficiency of exploitation of heterosis at the commercial level increased due to the availability of more cytoplasmic genetic male sterile sources for fertility restoration. Dominant fertility restoring nuclear genes are transmitted from the male parent, which allows seeds to 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 [3-4]. 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 the fertility restoration system in sorghum using F_2 and BC_1F_1 generations in three sorghum hybrids carrying A_1 cytoplasm.

Methodology

Cytoplasmic genetic male sterile line (296A) crossed with 238 RILs (Recombinant Inbred Lines) individuals (296B x IS 18551) to produce F_1 hybrids, evaluated in rainy and post-rainy season 2016 for seed setting percentage and pollen fertility/viability score. From this 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 the genetics of fertility restoration based on 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 F_1 plants to their male sterile line (296A) to produce BC_1 seeds. The F_2 's and BC_1 s along with their parents were raised at International Crops Research Institute for Semi-Arid Tropics, Patancheru, India during the late post-rainy season 2017-18. Before 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. Based on seed setting percentage, the F_2 and BC_1 are classified into fertile, partial fertile, and sterile [5]. The F_2 and BC_1 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 F_2 and BC_1 generations was tested using the chi-square test [3-4, 6-7].

Statistical analysis

All the data analysis was carried out in R software version 4.1.3 [8]. Chi-square (χ^2) method with Yates correction factor [9] was applied to 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 levels. The null hypothesis was rejected if the calculated χ^2 value exceeded the corresponding tabulated χ^2 value. The 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

∑ = summation over all classes

n = number of independent classes in the hypothetical distribution

Results and Discussion

The inheritance of the A₁ CMS system was investigated on F₂ and BC₁ in a total of 3 (A x R) crosses. All the F₂ and BC₁s 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 [5]. The results of the inheritance of the A₁ CMS system for 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 BC₁ 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 BC₁ plants divided into 301 fertile (F) and 284 sterile (S) show segregation according to a 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).

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)

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

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, the mechanism of action has not been determined definitively for any restorer allele. The knowledge of fertility restoration genetics is of utmost importance for the transfer of restorer genes from one genotype to another [10]. 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 produces male sterility [11]. 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 the 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 [12]. 3 F₂s and 3 BC₁s 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 nonsignificant χ^2 values concluding that observed and expected values are very negligible and/or no differences [10, 13-17]. The results are discussed below. In six segregating populations (3 F₂s and 3 BC₁s) developed from three crosses, a monogenic F₂ ratio of 3F:1S and the corresponding BC₁ ratio of 1F:1S was observed the results from a single dominant gene involved in fertility restoration. Consider the 'A' gene involved in fertility restoration of the A₁ CMS system. The monogenic F₂ ratio of 3F:1S and the corresponding BC₁ ratio of 1F:1S is possible when the genotype of the female parent is 'aa' and of restorer parents is 'AA' indicating that a single dominant gene is involved in fertility restoration. The F₁ of these parents will be fertile and heterozygous for a single locus (Aa). A plant in the F₂ will be fertile if it possesses a dominant allele of the basic gene/s. All other plants will be sterile. In backcross, the F₁ plant (Aa) crossed with a female parent (aa). From this, half of the plants containing the 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 (milo) CMS system [7, 4, 17-18], for A₂ [4], for maldandi cytoplasm [3] and for 9E and A₄ CMS systems [19]; in pearl millet for A₄ CMS system [20] and in A₅ [21] in rice for CMS-BT [22], CMS-HL [23], in sunflower CMS-PET1 [24-25] and for CMS-ANL2, CMS-PEF1 and CMS-PET2 [24-25], in rice [16, 26], in Brassica [27], in pigeon pea [10].

Conclusion

It is necessary to keep upgrading the hybrid technology so that high-yielding hybrids can be developed. The knowledge of 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 percentages can be selected.

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