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RESEARCH ARTICLE

Understanding the number of genes governing fertility restoration and isolation of potential restorers on *maldandi* source of male sterility in *rabi* sorghum [*Sorghum bicolor* (L.) Moench]

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Abstract

DSMR-4 and DSMR-8, two promising restores, were crossed with M 31-2A maldandi cytoplasm. The obtained hybrids were assessed in three sets of tests, F_2: BC_1F_1, F_2: F_3, and F_4 kharif: F_4 rabi generation, in order to determine the number of genes driving the segregating pattern. Three genes are involved in the initial set of segregation patterns for both crosses: in the F_2 generation, there were 54 fertile: 10 sterile and in the BC_1F_1 generation, there was 1 fertile: 1 sterile. Two of these genes are required for the restoration and function in a duplicate complimentary manner. Further, both the crosses were grown in F_3 generation to confirm the stability of restorers. They were segregated in the ratio of 10 true-breeding families and 44 segregating families, which confirmed F_2 ratio. Further, only true breeding 49 to 54 families of both the crosses were forwarded to F_4 generation and evaluated in both *kharif* and *rabi* seasons. In both seasons, they segregated in the ratio of 4 true-breeding families and 6 segregating families. The current study revealed the reliability of gene action for fertility restoration increased from F_2 to F_4 because of the fixation of alleles.

Keywords: *Maldandi* cytoplasm, seed set percent, gene action, restoration pattern.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench], being a C_4 plant with higher photosynthetic efficiency and abiotic stress tolerance (Reddy et al. 2009) adapted to a diverse set of environments ranging from arid and semiarid to tropical regions throughout the world. On a global basis, sorghum is the fifth major cereal crop in the extent of production following wheat, rice, maize, and barley. Along with providing food and fodder, it plays a vital role in providing micronutrients at a low cost (Rao et al. 2010) as it is necessary for a country like India, having 25% of the population is poor and which stood in a serious category in Global Hunger Index 2023, positioning 111th place out of 121 countries. So, this crop addresses the issues of climate change, malnourishment and to some extent, poverty through its sustainable "low cost and more micronutrient" phenomenon.

CMS in sorghum was first discovered when the nuclear genome of *kafir* was moved into an incompatible cytoplasmic background of *milo*. This discovery of A_1 CMS and its subsequent exploitation for hybrid production has revolutionized sorghum production because F_1 hybrids are superior by 50-60% in their grain yield compared to the traditional landraces. Of the several cytoplasm available in sorghum, only A_1 (*milo*) CMS system is predominantly used for commercial production of hybrids (Reddy et al. 2010), though it lacks good grain quality. In addition to the A_1 cytoplasm, several other CMS sources like A_2, A_3, A_4,

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Indian A_4, A_5, A_6, 9E, and KS were described in sorghum. Among these *maldandi* (A_4) cytoplasm is known for its grain quality with shoot fly resistance. However, *maldandi* cytoplasm faces a paucity of promising restorers and their stability (Kariyannanavar et al. 2023). Identification of such restores will help to broaden the genetic base and exploit hybrid vigor. In the present study, an effort has been made to identify the number of genes governing and inheritance of fertility restoration among hybrids developed by crossing restores on *maldandi* cytoplasm in an early segregating generation. Further, an attempt was made to isolate potential restorers in the segregating generation.

Material and methods

The experimental material comprised of a male sterile line (M 31-2A) and two restorers (DSMR-8 and DSMR-4) reported by Verma et al. 2022. Each of these restorers crossed with M 31 2A in *kharif* 2018 and the obtained F_1's were forwarded to F_2 and simultaneously backcrossed with a male sterile line to generate BC_1F_1 in *rabi* 2018. In one set, obtained seeds of F_2 and BC_1F_1 progenies were sown during the post-rainy season of 2019-20 at Botany garden MARS, Dharwad. Some F_2 seeds were kept as remnants and randomly selected F_2 plants were forwarded to the F_3 generation. In another set, remnant seeds of F_2 and randomly selected 250-270 F_3 families of each hybrid were grown in the post-rainy season of 2020. Further fertile true breeding 49-54 F_3 families of each hybrid were forwarded to F_4 generation and evaluated in the *kharif* and *rabi* 2021. For all the generations *viz.,* F_2, BC_1F_1, F_3 and F_4 recommended agronomic practices were followed to raise a good crop. Seeds of each generation were sown in a single plant per hill with a spacing of 15 cm between plants and 60 cm between rows. Each plant of all generations was labeled and before flowering at about the boot leaf stage when the panicle emerged, panicles were covered with a paper bag to avoid cross-pollination. Around 25 to 30 days after flowering (physiological maturity), each panicle was observed visually for seed set under selfing. Selected panicles were harvested and observed physically for the seed set, and the percent seed set was calculated using the following formula (Kishan and Borikar 1989).

Based on the seed set percent the genotypes were grouped into different categories of restoration. The χ^2 test for homogeneity of genetic ratios for a cross across the generation was done.

Results and discussion

Restoration in segregating and backcross generations

The F_1 plants of two crosses of the present study were fully fertile, which suggested that fertility restoration was dominant over sterility in *maldandi* cytoplasm systems. From a cross (M31-2A x DSMR-8), 672 F_2 plants were evaluated, 550 were fertile and 122 were sterile. Similarly, in BC_1F_1 out of 440 plants evaluated 235 were fertile and 205 were sterile. This gave a good χ^2 fits of trigenic expected ratio of 54:10 in the F_2 and 1:1 in the BC_1F_1 generations. Along with this, another cross (M31-2A x DSMR-4), gave the same results in F_2: Out of 560 plants, 466 were fertile and 94 were sterile. In support of this BC_1F_1 showed 1:1 segregation. Of 490 plants evaluated 261 were fertile and 229 were sterile (Table 1). In F_2 generation, out of three genes A, B, and C, any two dominant genes are necessary for fertility restoration (A_B_C, A_B_cc, aaB_C, and A_bbC_) however presence of a single dominant gene showed sterility (A_bbcc, aaB_cc, and aabbC_) this led to the segregation ratio of 54F:10S. To confirm this pattern same crosses were backcrossed to M31-2A, the obtained BC_1F_1 (AaBbCc X aabbcc) plants having a genetic constitution of at least two of the three dominant genes given fertile plants (A_B_C_, A_B_cc, A_bbC_ and aaB_C_) and plants having single dominant gene or all the recessive genes yielded to sterile plants (A_bbcc, aaB_cc, aabbC, and aabbcc) this showed the segregation ratio of 4 fertile and 4 sterile genotypes out of 8 genotypes (1F:1S) which endorsed the F_2 pattern of segregation as mentioned above. This confirmed the involvement of three dominant genes in the restoration of fertility; among them only two dominant genes are necessary for the restoration. This indicates that three genes are acting in a duplicate-complementary manner and endorsed by the earlier findings of Reddy et al. (2010).

Restoration in early segregating generations

Similarly, another set of F_2 plants involving the same crosses was evaluated in a separate experiment. The results in F_2 generation of the cross M31-2A x DSMR-8 followed a similar trend of segregation and gave a good χ^2 fits of 54:10 (367 fertile and 76 sterile out of 443 plants). In another cross M31-2A x DSMR-4, out of 451 F_2 plants, 371 were fertile and 80 were sterile and followed the same trend. The repeatability of the experiment increased the reliability of the inheritance pattern over the season. To confirm it more accurately randomly selected F_2 of each hybrid was forwarded to F_3. In a cross (M31-2A x DSMR-8), out of 250 F_3 families, 49 were true-breeding fertile, 201 were segregating and in another cross (M31-2A x DSMR-4), out of 270 F_3 families 54 fertile were true-breeding and 216 were segregating (Table 2). Further, only true breeding 49 to 54 families were forwarded to F_4 generation and evaluated in both *kharif* and *rabi* seasons. In *kharif*, a cross (M31-2A x DSMR-8), out of 49 F_4 families, 23 were true-breeding fertile, 26 were segregating and in another cross (M31-2A x DSMR-4), out of 54 F_4 families 24 were fertile true-breeding and 30 were segregating. Interestingly, both the crosses showed a similar trend in *rabi* also (Table 3).

The F_3 families of both crosses segregated in the ratio of 10 fertile true-breeding to 44 fertile segregating. The F_3 families bearing a genetic constitution of at least two

dominant genes were fertile true-breeding (AABBC_, A_BBCC, AAB_CC, AABBcc, AAbbCC and aaBBCC) and the other families having heterozygosity for one gene, two-gene, and three genes got segregated within the family. Thus segregation pattern led to 10 fertile true breeding and 44 fertile segregating families out of 54 genotypic classes rather, it would be 64 genotypic classes because 10 sterile genotypic classes were eliminated through the process of selection in F_2 itself. The Segregation pattern within a family was difficult to conduct because, at least for single gene segregation minimum of 64 plants were necessary as the current experiment had hardly 20 plants in a family so it was beyond the scope of the current experiment to note the number genes segregating within the family since the segregation within a family may be for one or two or three genes (Kumar et al. 2004). By and large, it was evident that there was segregation within the family and this confirmed the involvement of three dominant genes in the restoration of fertility by endorsing F_2 pattern of segregation. Jordan et al. (2010) and Reddy et al. (2010) also documented three major restorer genes *viz., Rf*1, *Rf* 2 and *Rf* 5 for fertility restoration in sorghum.

Further, F_4 families of both crosses segregated in the ratio of 4 fertile true breeding and 6 fertile segregating. The F_4 families were bearing a genetic constitution of two homozygous dominant and single homozygous recessive genes shown fertile true breeding (AABBCC, AABBcc, AAbbCC and aaBBCC) and the other families having heterozygous for one gene got segregated within the family. In the next generation there won't be segregation within a family and between families because there will be no heterozygous restorer genes. This makes the selection of promising restorer easier in F_4 onwards as compared to F_2 because of the fixation alleles and rejection of fertile segregating families in the previous generations (Reddy et al. 2010). Thus, as the generation preceded from F_2 to F_4 the true breeding fertile family's ratio decreased from 54 to 4. In addition to this, F_4 material was evaluated in *kharif* and *rabi* season. There was no variation in the ratio between two seasons, implying the stability of the restoration. The accuracy of gene action confirmation for fertility restoration increases as we move from F_2 to F_6 because of the fixation of allele. In this experiment, both the restorers (DSMR-4 and DSMR-8) have shown similar and stable inheritance patterns on *maldandi* cytoplasm over the generation (54:10 in F_2, 1:1 in BC_1, 10:44 in F_3 and 4: 6 in F_4) despite the involvement of minor genes and modifier. Beyond this, the reliability of stable restores and their pattern of inheritance in the present

Table 1. Segregation ratios for fertile and sterile plants in F_2 and back cross population derived from cross involving maldandi cytoplasm-based A line and stable restorer

Cross	Generation	Total no. of plants	Fertile plants (F)	Sterile plants (S)	Expected ratio (F:S)	Calculated χ^2	Table χ^2 at df = 1
							0.05
M 31-2A × DSMR-8	F_2	672	550	122	54:10	3.26	3.84
	BC_1	440	235	205	1:1	2.04	3.84
M 31-2A × DSMR-4	F_2	560	466	94	54:10	0.57	3.84
	BC_1	490	261	229	1:1	2.08	3.84

Table 2. Segregation ratios for fertile and sterile plants in F_2 and F_3 populations derived from cross-involving maldandi cytoplasm-based A line and stable restorer

Cross	F_2 Segregations					F_3 Segregations			
	Total no. of plants	Fertile plants (F)	Sterile plants (S)	Expected ratio (F:S)	Calculated χ^2	No. of families tested	True breeding fertile families	Families segregating	χ^2 10:44 F: Segregation
M 31-2A × DSMR-8	443	367	76	54:10	0.78 (ns)	250	49	201	0.23 (ns)
M 31-2A × DSMR-4	451	371	80	54:10	1.53 (ns)	270	54	216	0.39 (ns)

Table 3. Segregation ratios for true breeding v/s segregating F4 families in *kharif* and rabi season

CROSS	Generation	No. of Families	True breeding fertile families	Families segregating	χ^2 4:6 F: Segregation	Table χ^2 at d f =1	
						0.05	*0.01*
M 31- 2A × DSMP-8	F_4 *kharif*	49	23	26	0.98 (ns)	3.84	6.63
	F_4 *rabi*	49	15	34	1.79 (ns)	3.84	6.63
M 31- 2A × DSMP-4	F_4 *kharif*	54	24	30	0.44 (ns)	3.84	6.63
	F_4 *rabi*	54	21	33	0.02 (ns)	3.84	6.63

crosses is also confirmed by validating at the molecular level for fertility restoration (*Rf1, Rf2, Rf3, Rf4, Rf5, Rf6*) reported by Kumar et al. (2023).

Authors' contribution

Conceptualization of research (PK, BDB); Designing of the experiments (PK, LKB, VSK, RSP); Contribution of experimental materials (BDB, RSP, AR); Execution of field/ lab experiments and data collection (PK, GG, NPK, AR, RSP); Analysis of data and interpretation (PK, BDB, LKB, AR, NPK); Preparation of the manuscript (PK, BDB, AR, GG, RSP).

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References

Arunkumar B., Biradar B. D. and 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. Plant Breed., **64**(4): 325-326.

Jordan D. R., Mace E. S., Henzell R. G., Klein P. E. and Klein R. R. 2010. Molecular mapping and candidate gene identification of the Rf2 gene for pollen fertility restoration in sorghum (*Sorghum bicolor* (L.) Moench). Theor. Appl. Genet., **120**: 1279–1287.

Kariyannanavar P., Wali M.C., Biradar B.D., Kubsad V.S., Verma L.K., Kumar N.P. and Revanasiddayya. 2023. Identifying B and R lines on maldandi Cytoplasm besides Assessing their Genetic Diversity in *rabi* Sorghum [*Sorghum bicolor* (L.) Moench]. Indian J. Genet. Plant Breed., **83**(3): 374-379.

Kishan A. G. and Borikar S. T. 1989. Genetic relationship between some cytoplasmic male sterility systems in sorghum. Euphytica, **42**: 259-269.

Kumar P.N., Biradar B. D., Bagewadi B., Hanamaratti N. G., Bhat S., Shekharappa, Nethra P., Kariyannanavar P. and Kavyashree N. M. 2023. Identification of SSR markers linked to new fertility restoration trait in sorghum [*Sorghum bicolor* (L.) Moench] for A4 (*maldandi*) male sterile cytoplasm. Plant Breed., 1–9. https://doi.org/10. 1111/pbr.13155

Rao P., Basavaraj P. G., Bhagavatula S. and Ahmed W. 2010. An Analysis of availability and utilization of sorghum grain in India. J. SAT Agric. Res., **8**: 1-6.

Reddy B. V. S., Ramesh K.S., Reddy P. S. and Kumar A. A. 2009. Genetic enhancement for drought tolerance in sorghum. Plant Breed. Rev., **31**: 189-222.

Reddy P. S., Rao M. D., Reddy B. V. S. and Kumar A. A. 2010. Inheritance of male sterility restoration in A_1, A_2, A_3 and $A_{4(M)}$ cytoplasmic male-sterility systems of sorghum [*Sorghum bicolor* (L.) Moench]. Indian J. Genet. Plant Breed., **70**(3): 240-246.

Verma L. K., Biradar B. D., Patil S. S., Chattannavar S. N. and Kubsad V. S. 2022. Comparative analysis of restoration behavior of milo (104A, 401A) and maldandi (M31-2A) based male sterile lines in sorghum. Genetic Resou. Crop Evol., **69**: 559-566.