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Inheritance Pattern of A1 Cytoplasmic-nuclear Male Sterility System in Commercial Pearl Millet Hybrids [Pennisetum glaucum (L). R. Br.] in Summer and Post-rainy Seasons

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ABSTRACT

Background: This study aimed to understand the genetic aspects and inheritance patterns that control the process of restoring fertility in the context of the A1 system of Cytoplasmic-Nuclear Male Sterility (CMS) in pearl millet.

Methods: The experimentation was conducted in commercial pearl millet hybrids, utilizing three F2 populations and three BC1 populations. These populations underwent assessment during the rainy and post-rainy seasons at Rallis India Limited, Hyderabad During the Post-rainy (November-February) and summer (March-June) seasons of 2022-2023. Segregation data derived from observations on pollen shedding and seed set were gathered and subsequently, statistical analysis was performed. The Chi-square (χ^2) test was employed to assess the conformity of the observed segregation data to the expected genetic ratios, thus evaluating the goodness of fit.

Result: The hybrid combinations MP13P025A × MP16P050R (MP7171) and MP18P069A × MP20P081R (MP7214) consistently displayed a 3:1 segregation ratio in the F2 generation and a 1:1 ratio in the BC1 generation, regardless of the rainy or post-rainy seasons. However, the cross between MP14030A and MP15P038R (MP7878) showed irregularities in both seasons due to modifiers and environmental factors. These irregularities stemmed from an excess of fertile plants (FF) in the post-rainy season or an excess of sterile plants (FS) in the summer season. These observations provide valuable insights into the genetic dynamics of the A1 Cytoplasmic-Nuclear Male Sterility (CMS) system within commercial pearl millet hybrids. The study indicates that fertility restoration in the A1-based CMS system is strongly influenced by a single Dominant gene control mechanism governing the interplay between male sterility and fertility restoration in these crosses.

Key words: A1 cytoplasm inheritance, A1 cytoplasm, Inheritance, Male fertility restoration, Pennisetum glaucum.

INTRODUCTION

Pearl millet (Pennisetum glaucum L.), a hardy cereal crop, is cultivated in challenging conditions such as scanty and erratic rainfall, elevated temperatures and low soil fertility. This resilient crop stands as an essential source of food and cattle feed for agricultural communities in arid and semiarid regions of sub-Saharan Africa and South Asia. Pearl millet is cultivated across over 27 million hectares in Africa's arid and semiarid tropical regions (17 million ha) and Asia (10 million ha). The significance of pearl millet cultivation footprint impacts the lives of over 90 million people in these regions, where it constitutes a dietary fiber. In India, it ranks as the fourth most extensively cultivated cereal food crop, followed by rice, wheat and maize. Impressively, during the 2020-21 period, pearl millet was cultivated on 7.41 million hectares, yielding a total production of 10.3 million tons with an average yield of 1391 kg per hectare. Key states, including Rajasthan, Uttar Pradesh, Maharashtra, Haryana and Gujarat, significantly contribute to over 90% of the cultivated area, accounting for 87.7% of the overall production (Satyavathi, 2019). Beyond its yield, pearl millet's nutritional impact is equally remarkable, providing an estimated 80-90% of the calorie intake for millions of underprivileged individuals globally (Burton et al., 1972). Notably, in India, this cereal plays an essential role in rural

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diets, particularly in regions like western Rajasthan and Gujarat, is comprising more than half of the cereal consumption. India, the country with the highest per capita consumption by a rural population, especially in western Rajasthan and Gujarat, contributes more than 50% of the cereal consumption in these regions (Rao et al., 2006). Since the 1960s, pearl millet improvement programs in India have been mainly based on developing hybrids.

The importance lies in the immense global relevance of pearl millet cultivated across arid and semiarid regions of

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sub-Saharan Africa and South Asia. The pearl millet is a lifeline for communities with challenging climatic conditions and limited resources, food sources, cattle feed and livelihood for over 90 million people, The crop's productivity and resilience are vital to mitigating hunger and enhancing economic stability in these regions. The average productivity of pearl millet is from 305 kg/ha in the 1950s to the present yield of 1391 kg/ha. In India, hybrids are cultivated in about 60 to 70% of the area (~5.0 m ha) (Satyavathi 2017). The importance of hybrids in pearl millet in India can be cited because hybrid adoption has led to an acreage increase of about 300% in crop productivity since 1951 (Yadav *et al.*, 2015)

The discovery of the A1 cytoplasmic, nuclear male sterility system and breeding Since the first commercial single cross hybrid development in 1965, most hybrids are based on the A1 CMS system. However, even after more than 58 years of extensive utilization of the A1 CMS system, there are not many reports on the genetics of this system. Burton and Athwal (1967) hypothesized a single recessive gene responsible for male sterility and its dominant allele for male fertility restoration. However, Siebert (1982) suggested two dominant complementary genes with at least one modifier to govern male fertility restoration in the A1 cytoplasm. Yadav et al. (2010) observed different results in twelve other crosses and suggested a single-gene control, two-gene control, or three-gene control model with two duplicate complementary loci. Chandra et al. (2022) confirms that the different digenic ratios of 9:7 and 1:3 of fertile and sterile plants were observed in F2 and BC1 generations, indicating a complementary interaction of two genes. All the previous studies for A1 inheritance were based on only the pollen shedding criterion and the summer and rainy seasons; pollen shedding and seed set for commercial hybrid-based investigations and understanding the genetics of A1 CMS were not exploited. This study aimed to comprehensively understand the inheritance pattern governing male sterility and fertility restoration in pearl millet. Pollen shedding and seed set percentage data, combined with commercially released hybrids and their diverse F2 and BC1 generations, were meticulously evaluated across two seasons, such as post-rainy and summer.

MATERIALS AND METHODS

The plant experimental material consisting of three diverse Cytoplasmic A-lines possessing A1 cytoplasm contains three diverse genetic backgrounds and restorers of diverse parentage, as presented in Table 1. The three A1 lines (MP13P025A1, MP14P030A1 and MP18P069A1) were developed by more than seven backcrosses of MP13P025B, MP14P030B and MP18P069B and selected corresponding A1 CMS lines developed internally. All three hybrids tested different targeted geography and commercially released names (MP7171, MP7878 and MP7214) were crossed with corresponding restore parents to produce the three F1 hybrids. Experimental materials are described in detail in Table 1.

Individual plants were used for making plant × plant crosses to produce these F1s. More than ten plants from each F1 were selfed to produce three F2 populations. Bulk pollen from 5-10 plants from each F1 was used to cross on the respective parental A-lines to produce BC1 populations. Field trials of these 6 parents, 3 F1s, 3 BC1s and 3 F2s, were conducted at the Rallis India Limited R&D Research Center at Hyderabad Station, Telangana, India, during the Post-rainy (November-February) and summer (March-June) seasons of 2022-2023. The mean maximum and minimum temperatures during this period in the post-rainy season were 24°C (range 24.3-27.5°C) and 30°C (range 26.8-33.6°C), respectively. During the post-rainy season, the mean relative humidity at 0700 hours was 75% (range 40-95%) and at 1400 hours, it was 35% (range 14-69%). During the rainy season, the mean relative humidity at 0700 hours was 88% (range 80-98%); at 1400 hours, it was 61% (range 48-76%). The parents, F1's, were grown in single-row plots of 4m length plants, with approximately 30-35 plants per plot, at Rallis RandD Station, Hyderabad. Each F2 population was evaluated in twelve-row plots of 4 m length with approximately 300-350 plants per plot and each BC1 population was raised in eight rows of 4 m length with about 240-280 plants per plot. Pollen shedding of individual plants was used to determine the male fertility (FF) and sterility (FS) reaction of individual plants in all the populations. Plants were scored for pollen shedding between 0800 and 1100 hours by tapping the inflorescence and observing for pollen shed. Those shedding pollen were scored as fertile (FF) and non-shedders as sterile (FS), and seed set percent evaluation was performed in all seasons in BC1 and F2 populations. One head of each of the plants was bagged for seed set. Scoring and classifications of sterile and fertile plants based on seed set% and Pollen shed criteria are described in detail in Table 2,3.

Based on the scoring, the sterile and fertile groups were categorized, with a score of 0,1 for the sterile group and above 7 to 9 for the fertile group. By doing so, we gain confidence in the genetic ratio, which gives us double confirmation of the segregation ratio of fertility restores genes for both the Post-rainy and rainy seasons. Pictures of pollen shed and seed set% were taken during the Flowering and Maturity stage in BC1 and F2 population of MP7171 (Fig 1).

Statistical analysis

The collected segregation data from pollen shedding and seed set data during the post-rainy and summer seasons were subjected to statistical analysis using the Chi-square (X^2) test, which was applied to the observed segregation data to test the goodness of fit on probable genetic ratios.

RESULTS AND DISCUSSION

All the F1 plants of three crosses were fully fertile under bagging conditions, as complete pollen shedding and successful seed set were observed. These results strongly indicate the dominant nature of the fertility restoration gene(s) in the A1 cytoplasm-based genetic male sterility system. The pollen shedding and seed set data of all F2s and BC1s as well as the χ^2 value for the crosses of Three CMS lines (MP13PO25A, MP14P030A and MP18P069A) and Restorer lines (MP16P050R, MP15P038R and MP20P081R) are described in detail in Table 4, 5.

In the present study, the cross MP13P025A X MP16P050R(MP7171) showed a segregation pattern of 210 male-fertile and 70 male-sterile plants during the summer season based on seed set. The observed segregation pattern fits well with the expected 3F:1S ratio with a χ^2 probability of 0.79, suggesting that a single gene control system is responsible for restoring male fertility in this cross. Additionally, in the BC1 generation, the segregation pattern closely followed the expected 1F:1S ratio with a χ^2 probability of 0.46, further supporting the hypothesis of a single-gene control system. The results of the same cross combination, MP13P025AXMP16P050R (MP7171), exhibited interesting segregation patterns during the Post-rainy season. The F2 generation fit the expected 3F:1S ratio for both seed set data (χ^2 probability = 0.69) and pollen shedding data (χ^2 probability = 0.47). However, in the BC1 generation, the segregation did not fit the expected 1F:1F ratio for pollen shedding data (χ^2 probability = 0.01), although it maintained a good fit for seed set data (χ^2 probability = 0.11).

According to the proposed hypothesis, the restorer genes (AA) are single dominant alleles at the A locus, while the recessive gene for sterility is represented by (aa). In this scenario, the segregation pattern observed in the crosses indicates that male-fertile plants could be either heterozygous (Aa) or homozygous (AA) for the dominant restorer allele, falling in the fertile group. The male-sterile plants would be homozygous (aa) for the recessive allele, placing them in the sterile group. The F1 generation will inherit the dominant allele from the Rline and the recessive allele from the female line, resulting in heterozygous (Aa) plants. In the F2 generation, the expected segregation ratio will be 3F:1S due to the presence of heterozygous plants (Aa) and homozygous dominant plants (AA) in the fertile group and homozygous recessive plants (aa) in the sterile group. The frequency of encountering 2-gene segregation patterns or 1-gene inheritance patterns in pearl millet Germplasm largely depends on dominant.

Restorer alleles at A locus in the breeding population. If the frequency of dominant restorer alleles is high, most crosses will likely exhibit the observed 3F:1S and 1F:1S segregation ratios, in line with the single-gene control system. However, if multiple loci are involved in male fertility restoration, 2-gene or more complex segregation patterns may be observed in certain crosses.

The cross-combination of MP14030AXMP15P038R (MP7878), a commercial hybrid, exhibited inconsistent segregation patterns in both the F2 and BC1 generations, indicating issues with the genetic backgrounds of the materials involved. During the summer season, the

segregation pattern in the F2 generation did not fit the expected 3F:1S ratio for both seed set and pollen shedding data. The χ^2 probability of 0.00 and 0.00 is a poor fit to the expected ratios. This suggests that the genetic factors responsible for male fertility restoration in this cross did not follow the Mendelian inheritance pattern during the summer. The BC1 generation during the summer season, the segregation pattern did fit the expected 1F:1S ratio for both the seed set and pollen shedding data, with χ^2 probability values of 0.11 for the seed set and 0.17 for pollen shedding. This indicates a relatively good fit, although slight deviations from the expected ratios exist. In the Post-rainy season, the same cross combination of MP14030AXMP15P038R (MP7878) continued to exhibit inconsistent segregation patterns in both the F2 and BC1 generations, indicating persistent issues with the genetic backgrounds of the materials involved.

In the F2 generation during the post-rainy season, the segregation pattern did not fit the expected 3F:1S ratio for both seed set and pollen shedding data. The χ^2 probability is 0.00;a similar pattern was also observed during the summer season. This suggests that the genetic factors responsible for male fertility restoration, in the BC1 generation during the post-rainy season, the segregation pattern also did not fit the expected 1F:1S ratio for both seed set and pollen shedding data, with χ^2 probability values of 0.00.This indicates a complete inconsistency in the segregation patterns observed in F2 and BC1 generations during the post-rainy season.

To tackle these challenges, a comprehensive analysis of the genetic backgrounds of the parental lines is essential. This examination aims to identify any elements within the

Table 1: Pedigree of experimental material used in A1 CMS gene inheritance studies.

Туре	Hybrid name	Pedigree
Hybrid	MP7171	MP13P025A/MP16P050R
Hybrid	MP7878	MP14P030AXMP15P038R
Hybrid	MP7214	MP18P069AXMP20P081R

Table 2: Classification of sterile and fertile genetic ratios in theselfed bag based on seed set %.

Score	Classification	Category
0	100% sterile plants	FS
1	1-5 seeds in selfed bag	FS
7	Fertile plants	FF
8	Fertile plants	FF
9	Fertile plants	FF

Table 3: Classification of sterile and fertile genetic ratios in selfed bag based on Pollen shedding.

Score	Classification	Category
No shedding	100%s sterile plants	FS
Pollen shedding	Fertile plants	FF

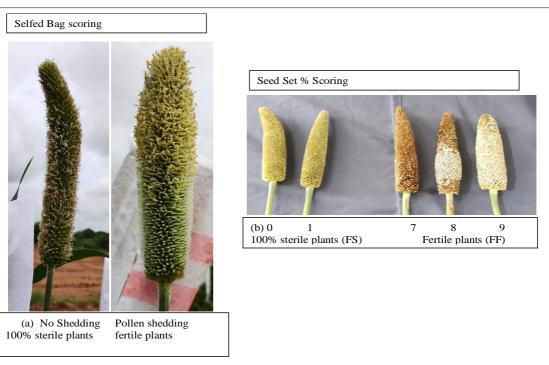
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genetic makeup that could influence the inconsistent restoration of male fertility. Additionally, a deeper investigation is warranted to uncover the potential genetic or environmental factors responsible for the deviations observed in the segregation patterns within this cross-combination. Further research is imperative to grasp better the intricate interplay between genetics and the environment, which could provide insights into the mechanisms underlying the observed variations.

In the cross combination MP18P069AXMP20P081R (MP7214), a commercial hybrid showed a good fit to the expected ratios in both the F2 and BC1 generations, indicating a single gene responsible for fertility restoration during the summer season. The segregation pattern in the F2 generation fitted well with the expected 3F:1S ratio for both seed set and pollen shedding data. The χ² probability values were 0.30 for the seed set and 0.65 for pollen shedding, indicating a relatively good fit to the expected ratios. In the BC1 generation during the summer season, the segregation pattern also showed a good fit to the predicted 1F:1S ratio for both seed set and pollen shedding data. The χ^2 probability values were 0.31 for the seed set and 0.60 for pollen shedding.

Similarly, in the post-rainy season, the segregation pattern in the F2 generation continued to fit well with the expected 3F:1S ratio for both seed set and pollen shedding data. The χ^2 probability values were 1.00 for the seed set and 0.46 for pollen shedding, indicating a consistent male fertility restoration pattern. In the BC1 generation during the post-rainy season, the segregation pattern also fit the expected 1F:1S ratio for both seed set and pollen shedding data. The χ^2 probability values were 0.67 for the seed set and 0.53 for pollen shedding data.

The results of the study indicate that the overall segregation pattern of male-sterile (FS) and male-fertile (FF) plants in populations derived from crosses between stable A-lines (MP13P025A and MP18P069A) and diverse R-lines (M16P060R and MP20P081R) suggests a single gene control system for male sterility and fertility restoration. The observed segregation ratios in the F2 and BC1 populations generally gave a good χ^2 fit with the expected 3F:1S and 1F:1S ratios, respectively. Out of 3 cases of F2s from these crosses (2 F2s evaluated in two seasons for pollen shedding and seed set data), segregation patterns followed the expected ratios. In one of the F2 populations, during the summer season, post-rainy, an excess of male-sterile and fertile plants were observed. The only cases that (in



(a) Some plants do not shed pollen in both BC1 generations of the MP7171 populations. They are considered "Sterile Plants". Plants that do shed pollen are considered "Fertile Plants". (b) Photographs were taken during the harvesting of mature heads of the plants in F2 generation of the MP7171 populations. Assessing the seed set percentage in selfed bags (bags containing the plant's pollen for self-fertilization). Plants based on their seed set percentage: 0 and 1: score indicate plants with very low or no seed set. These are considered "Sterile Plants." Score 7, 8 and 9: indicate plants with relatively higher seed set percentages and are considered "Fertile Plants".

Fig 1: Flowering and seed set percentage at harvesting stage.

Table 4: Fertility restoration gene (Rf) Segregation for male-fertile (FF) and male-sterile (FS) plants in F₂ and BC₁ generations and test of goodness of fit based on seed set data on

A1 CMS background of MP717, MP7878, MP7214 commercial	of MP717, MP78	78, MP7214 con		pearl millet hybrids, post rainy and summer 2022-2023.	ds, post rainy	and summer	2022-2023					
Pediaree	Variety	Season	Total	Generation	No. of plants observed	s observed	Expected ratio		No. of plants expected	expected	c	
	(5)		plants		ŦF	FS	FF	FS	분	FS	*	ı
MP13P025A/MP16P050R	MP7171	Summer	170	F2	126	44	က	-	128	42	0.07	0.79
			186	BC1	88	86	_	~	93	93	0.54	0.46
		Post rainy	308	F2	228	80	က	~	231	77	0.16	69.0
			227	BC1	108	119	_	~	113	113	0.53	0.47
MP14P030A×MP15P038R	MP7878	Summer	182	F2	120	62	က	~	136	45	7.98	0.00
			173	BC1	26	9/	_	~	86.5	86.5	2.55	0.11
		Post rainy	210	F2	138	72	က	~	157	53	99.6	0.00
			192	BC1	120	72	_	~	97.5	97.5	12.00	0.00
MP18P069A×MP20P081R	MP7214	Summer	263	F2	190	73	က	~	197	99	1.07	0:30
			167	BC1	06	77	_	~	83.5	83.5	1.01	0.31
		Post rainy	260	F2	195	65	က	~	195	99	0.00	1.00
			141	BC1	73	89	_	_	70.5	70.5	0.18	0.67
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FS=Full sterile, FF=Full fertile, **P=Probability.

Table 5: Fertility restoration gene (Rf) Segregation for male-fertile (FF) and male-sterile (FS) plants in F₂ and BC₁ generations and test of goodness of fit based pollen shedding data on A1 CMS background of MP717 MP7878 MP7214 commercial pearlmillet hybrids. Post rainy and summer seasons 2022-2023

on A1 CIVIS background of IVIP/11/, IVIP/18/8, IV	aorimi <i>P</i> 717, N	11P/8/8, MP/214	IP7214 commercial	pearimiliet nybrids, Post rainy and summer seasons 2022-2023.	ids, Post rainy	and summer	seasons zu	77-70	3.			
Dediaree	Variety	S S S S S S S S S S S S S S S S S S S	Total	(No. of plants observed	s observed	Expected ratio		No. of plants expected	expected	ç	ſ
	validiy		plants	Generation	世	FS	世	FS	뜐	FS	×	ı
PM12PP0023A×PMDG140541R	MP7171	Summer	140	F2	86	42	3	_	105	35	1.87	0.17
			170	BC1	83	87	_	_	85	85	60.0	0.76
		Post rainy	251	F2	169	82	3	_	188	63	7.87	0.01
			222	BC1	66	123	_	_	111	111	2.59	0.11
MP14P030A×MP15P038R	MP7878	Summer	197	F2	132	92	3	_	148	49	6.72	0.01
			136	BC1	09	9/	_	_	89	89	1.88	0.17
		Post rainy	240	F2	160	80	3	_	180	09	8.89	0.00
			144	BC1	86	46	_	_	72	72	18.78	0.00
MP18P069A×MP20P081R	MP7214	Summer	228	F2	168	09	3	_	170	28	0.21	0.65
			132	BC1	63	69	_	_	99	99	0.27	09.0
		Post rainy	240	F2	175	92	3	_	180	09	0.56	0.46
			160	BC1	92	84	_	_	80	80	0.40	0.53

FS=Full sterile, FF=Full fertile ,**P-Probability.

MP14P030AXMP15P038R) did not have a good fit to an expected 3F:1S ratio were all in the summer season, post-rainy for both pollen shedding and seed set data where an excess of male-sterile and fertile plants were observed. It did not fit well in any ratio due to inconsistency in genetic backgrounds. Similarly, out of three cases of BC1 from these crosses, all three BC1generations have a good fit to the expected 1F:1S ratio.

The deviations in segregation patterns could likely be due to environmental factors such as temperature and humidity playing crucial roles. The relatively lower temperatures and higher humidity in the post-rainy season might have promoted the expression of modifiers for fertility restoration, increasing in male-fertile plants. On the contrary, the higher temperatures and lower humidity in the summer have favored modifier expression for sterility, leading to more male-sterile plants.

It is essential to know that the effects of these modifiers are complex and may vary depending on the genetic backgrounds of the segregating populations, especially in the presence of major genes controlling male sterility and fertility restoration. This suggests that genetic background interacts with environmental factors to influence the segregation patterns of male sterility genes. The effects of these modifiers could be inconsistent, depending on the genetic backgrounds of the segregating populations with the major genes for male sterility and fertility restoration present. Similar Genetical studies in other crops such as maize (Zea mays) (Singh and Laughnan 1972), sorghum (Sorghum bicolor) (Tripathi et al., 1985), rice (Oryza sativa) (Govinda and Virmani, 1988), rapeseed (Brassica napus) (Pahwa et al., 2004), pepper (Capsicum annum L.) (Wang et al., 2004), pearl millet (Yadav et al., 2010) and Chandra et al., 2022 (Pearl millet) have shown a considerable effect of the genetic background and environments on the CMS inheritance. The segregation patterns observed in this study are more likely to arise due to a single gene control system.

According to the proposed hypothesis, the restorer genes (AA) are single dominant alleles at the A locus, while the recessive gene for sterility is represented by (aa). In this scenario, the segregation pattern observed in the cross indicates that male-fertile individuals could be either heterozygous (Aa) or homozygous (AA) for the dominant restorer allele, placing them in the fertile group. Similarly, male-sterile individuals would be homozygous (aa) for the recessive allele, placing them in the sterile group. To further explore the potential genetic basis, the R-lines would need to carry dominant alleles at the single locus at the same time, the female lines would possess the recessive allele at the same locus. This condition ensures that when the cross is made, the F1 generation will inherit the dominant allele from the R-line and the recessive allele from the female line, resulting in heterozygous (Aa) individual plants. When the F1 generation is self-pollinated (the F2 generation), the expected segregation ratio will be 3F:1S due to the presence of heterozygous individuals (Aa) and homozygous dominant individual plants (AA) in the fertile group and homozygous recessive individual plants (aa) in the sterile group. The observed segregation pattern in the BC1 generation, which fits the expected 1F:1S ratio, can be attributed to restoring male fertility due to the dominant allele (A) at the single locus in the R-line. The BC1 individual plants will inherit this dominant allele (A) from the R-line and either the dominant (A) or the recessive (a) allele from the female line. Consequently, the BC1 generation will consist of both heterozygous (Aa) and homozygous dominant (AA) individuals in the fertile group and homozygous recessive (aa) individuals in the sterile group; the segregation pattern observed in this study more likely due to single-gene control system, however, single-gene inheritance patterns will depend on the frequency of dominant restorer alleles at these single loci in pearl millet germplasm. In cases where the frequency of the restorer allele at one of the two loci is very low to rare, most studies produce the results of 1-gene inheritance under the 2-gene control system. The findings of our study are consistent with previous research conducted by Athwal (1965), Burton (1958) and Yadav et al. (2010), which also reported a single gene responsible for male sterility and fertility restoration in pearl millet. This single gene segregation pattern, observed in both F2 and BC1 populations, indicates that a dominant gene governs fertility restoration while a recessive gene controls sterility.

Moreover, Siebert (1982) and Chandra et al. (2022) likely reported segregation patterns related to male sterility and fertility restoration in pearl millet. These patterns may have previously reported the presence of two major dominant complementary genes involved in the fertility restoration of A1 cytoplasm in pearl millet. This observation highlights the complexity of the genetic control system for male sterility and fertility restoration in this crop. In line with previous studies, our research also supports the idea that genetic background and environmental conditions significantly influence the segregation patterns of male sterility and fertility restoration genes. Environmental factors such as temperature, humidity and seasonal variations may impact the expression of modifiers for fertility restoration and sterility, leading to deviations from the expected segregation ratios.

The variability and inconsistency observed in the segregation patterns, particularly when MP14030AX MP15P038R was involved as the female and male parent, emphasize the importance of using stable A-lines with well-defined male sterility characteristics in breeding programs. Unstable male sterility, as seen in MP14030A with pollenshedding behavior, can lead to excess fertile or sterile plants in subsequent generations, affecting the accuracy of genetic studies and hybrid development.

CONCLUSION

Our study further supports the presence of a single gene control system for male sterility and fertility restoration in pearl millet while acknowledging the potential influence of environmental factors on the expression of these genes. Future research could focus on allelism testing and the genetic mapping of fertility restorer genes. These investigations hold a promising understanding of the genetic architecture of male fertility in the A1CMS system. The Rf markers, also known as fertility restoration markers, hold significant promise for integration with genomic selection strategies in plant breeding programs. Several ways of benefiting include early Selection of plants, precision, reduced operational cost and data-driven decision-making. With genomic Selection, breeders can predict the performance of individual plants at early stages of plant development. Incorporating Rf markers into the prediction model makes identifying individuals with desirable fertility restoration traits possible even before they reach the flowering stage.

Conflict of interest

The authors have no conflict of interest.

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