Genetics of Fertility Restoration of the A4 Cytoplasmic Nuclear Male Sterility System in Pearl Millet

Mahalingam Govindaraj, *ICRISAT*

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The discovery of \( A_1 \) cytoplasmic-nuclear male sterility (CMS) at Tifton, Georgia, USA (Burton 1958) initiated the era of hybrid cultivar development in pearl millet \( \textit{Pennisetum glaucum} \) (L.). R. Br., which led to the release of the first grain hybrid in India in 1965 (Athwal 1965). Since then hundreds of commercial hybrids, all of them based on the \( A_1 \)-CMS system, have been developed and released or commercialized. This dependence on single cytoplasm makes the pearl millet hybrid seed industry vulnerable to disease and insect-pest epidemics, as witnessed in the case of southern leaf blight epidemic caused by \textit{Bipolaris maydis} race T on the Texas cytoplasm-based maize hybrids in the United States (Scheifele et al. 1970). This concern necessitated the search for new sources of CMS in pearl millet (Rai et al. 2006). Hanna (1989) identified an \( A_4 \) CMS system at Tifton, Georgia, USA in a wild grassy \textit{Pennisetum glaucum} (L.) R. Br. subsp. \textit{monodii} (Maire) Brunken. Also, an \( A_5 \) CMS system was identified in a pearl millet gene pool (Rai 1995). Among the various CMS systems reported so far, \( A_4 \) and \( A_5 \) CMS systems were found to have the most stable male sterility (Rai et al. 1996, 2001, 2006, 2009). Further, the frequency of maintainers is much higher for the \( A_4 \) CMS system than for the \( A_1 \) CMS system, and almost all lines are maintainers of the \( A_5 \) CMS system (Rai et al. 2006). Hence, these two CMS sources provide a much greater opportunity for the genetic diversification of A-lines, and thus a greater opportunity for diversifying the genetic base of hybrids provided more diversity is generated in the restorer lines. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) developed and disseminated 31 diverse and productive A-lines based on the \( A_4 \) CMS system during the period 1996–2004 (Rai et al. 2006). However, none of them was used by breeders in the public and private sector in India because of the paucity of restorers (R-lines) of this CMS system. Therefore, ICRISAT has now initiated a program focused on the development of \( A_4 \) CMS

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**Genetics of Fertility Restoration of the \( A_4 \) Cytoplasmic-Nuclear Male Sterility System in Pearl Millet**

**Shashi Kumar GUPTA, Kedar Nath RAI, Mahalingam GOVINDARAJ and Aluri Sambasiva RAO**

*International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India*

**Abstract**: Inheritance of fertility restoration of the \( A_4 \) system of cytoplasmic-nuclear male sterility in pearl millet was investigated using six crosses between two diverse male sterile lines (A-lines) and three diverse restorers (R-lines). The segregation pattern of male sterile (S) and male fertile (F) plants observed in \( F_2 \) and \( BC_1 \) in two seasons at ICRISAT, Patancheru, indicated the dominant single-gene control of male fertility restoration. The segregation pattern in \( BC_1 \) \( F_2 \) progenies derived from the fertile \( BC_1 \) plants evaluated for one season provided further evidence for the single-gene control. The season did not have much effect on fertility restoration. The information on the single-gene control of fertility restoration will help in diversifying the restorer genetic base of the \( A_4 \) CMS system and enhance R-line breeding efficiency in pearl millet.

**Keywords**: \( A_4 \) cytoplasm; fertility restoration; inheritance; male sterility; \textit{Pennisetum glaucum}
system restorers. It was observed that the fertility restoration ability of the A₄ CMS system restorers is less affected by the genetic background of the A-line than that of the A₁ CMS system restorers (Gupta et al. 2010). The understanding of the inheritance of fertility restoration of this CMS system can be useful in devising an effective breeding strategy. Except for a preliminary report (Du et al. 1996), there is not much information on the inheritance of fertility restoration of the A₄ CMS system. Therefore, the objective of this study was to undertake a more comprehensive study of the inheritance of fertility restoration of the A₄ CMS system in pearl millet using genetically diverse A- and R-lines.

MATERIAL AND METHODS

Plant material

The basic experimental material consisted of isocytoplasmic A-lines with A₄ cytoplasm in two diverse genetic backgrounds and three previously identified A₄-restorers of diverse parentage (Table 1). The two A₄-lines (81A₄ and ICMA₄ 88004) were developed by more than eight backcrosses of 81B and ICMB 88004, respectively, into A₄ cytoplasm.

Development of experimental populations

The two A-lines (81A₄ and ICMA₄ 88004) were crossed with each of the three diverse R-lines (IPC 511, IPC 804 and IPC 1518) to produce six F₁s in the 2009 rainy season. Single plants were used for making plant × plant crosses to produce these F₁s (81A₄ × IPC 511, 81A₄ × IPC 804, 81A₄ × IPC 1518, ICMA₄ 88004 × IPC 511, ICMA₄ 88004 × IPC 804, ICMA₄ 88004 × IPC 1518). The A-lines were maintained by crossing with their respective B-lines. In the post rainy season of 2009–2010, more than ten plants of each of the six F₁s were selfed to produce F₂ populations. Bulk pollen from five to ten plants from each F₁ was used to cross on the respective parental A-lines to produce BC₁ populations. Each F₁ was crossed with bulk pollen from the respective R-line to produce BC₂ population. During the 2010 summer season, panicles of five to seven fertile plants in each BC₁ were selfed to produce BC₁ F₂ progenies.

Field evaluation and data analysis

Field trials of five parents, six each of F₁s, F₂s, BC₁s, and BC₂s were conducted at ICRISAT, Patancheru, Andhra-Pradesh, India during the summer (March–June) and rainy (July–October) season of 2010, while BC₁ F₂ progenies were evaluated in the rainy season of 2010. The parents, F₁, BC₂ and BC₁ F₂ populations were evaluated in single-row plots of four meter length with mostly 25–35 plants per plot. Each F₂ population was evaluated in eight-row plots of four meter length with approximately 250–350 plants per plot, and each BC₁ population was evaluated in four rows of four meter length with about 125–150 plants per plot. Pollen shedding of individual plants was used to determine male fertility (F) and sterility (S) reactions of individual plants in all the populations. At full anthesis, plants were scored for pollen shedding between 08:00 and 11:00 h by tapping the inflorescence and observing for the pollen shed. Those shedding pollen were scored as male-fertile (F) and non-shedders as non-fertile (N).
male-sterile (S) (RAI & HASH 1990). Chi-square ($\chi^2$) test was applied to the observed segregation data in $F_2$, BC$_1$ and BC$_1$ $F_2$ populations to test the goodness of fit of various probable genetic ratios. The temperature and relative humidity were recorded from the 35$^{th}$ day to the 70$^{th}$ day of crop growth, which refers to one week prior to flowering of the first entry to one week after the last entry came to flowering in each season.

**RESULTS AND DISCUSSION**

Temperatures during the summer season 2010 ranged from 22.6 to 39.7°C (mean 30°C) and relative humidity at 07:00 h ranged from 65 to 92% (mean 77%). During the rainy season 2010, temperatures ranged from 21.1 to 29.4°C (mean 25°C) and relative humidity ranged from 94 to 96% (mean 95%). Thus, the two seasons of field trials represented two contrasting weather environments.

All the restorer parents (R-lines) had all plants fully fertile while the A-lines were fully sterile during both rainy and summer seasons. All the plants in six $F_1$s and the corresponding six BC$_2$s were also fully fertile during both the seasons, indicating fertility restoration for the A$_4$ CMS system to be controlled by dominant gene(s) that are in homozygous state in the present set of restorers. The $F_2$ population from the 81A$_4$ × IPC 511 cross segregated for 117 male-fertile (F) and 39 male-sterile (S) plants during the rainy season giving a perfect $\chi^2$ fit to a ratio of 3F:1S ($P = 1.00$) (Table 2), indicating the dominant monogenic control of fertility restoration. The BC$_1$ of this cross segregated for 78 male-fertile and 76 male-sterile plants during the rainy season and fitted well to the expected monogenic ratio of 1F:1S ($P = 0.46$). This segregation pattern repeated during the summer season with a good fit to the 3F:1S ratio in $F_2$ ($P = 0.96$) and 1F:1S ratio in BC$_1$ ($P = 0.46$). The $F_2$ from the 81A$_4$ × IPC 804 cross segregated for 210 male-fertile and 65 male-sterile plants during the rainy season and fitted well to the monogenic ratio of 3F:1S ($P = 0.60$). The BC$_1$ of this cross segregated for 92 male-fertile and 83 male-sterile plants and gave a good fit to the expected 1F:1S ratio ($P = 0.65$). This segregation pattern also repeated during the summer season with a good fit to the 3F:1S ratio in $F_2$ ($P = 0.32$) and the expected BC$_1$ ratio of 1F:1S ($P = 0.37$). In the 81A$_4$ × IPC 1518 cross the segregation pattern in both seasons had a good fit to 3F:1S ratio in $F_2$ and 1F:1S ratio in BC$_1$.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Season</th>
<th>Generation</th>
<th>No. of plants observed</th>
<th>Expected ratio</th>
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<th>$P$</th>
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<td></td>
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<td>$F$</td>
<td>$S$</td>
<td>$F$</td>
<td>$S$</td>
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<tr>
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<td>$F_2$</td>
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<td>39</td>
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<td>summer</td>
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<td>BC$_1$</td>
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<td>BC$_1$</td>
<td>69</td>
<td>77</td>
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</tbody>
</table>

$P$ – probability

Table 2. Segregation for male-fertile (F) and male-sterile (S) plants in $F_2$ and BC$_1$ generations and the test of goodness of fit for hypothetical Mendelian ratios in crosses of 81A$_4$ with the restorer parents IPC 511, IPC 804 and IPC 1518 in pearl millet, rainy and summer seasons 2010, Patancheru.
The F₂ from the ICMA₄ 88004 × IPC 511 cross segregated for 176 male-fertile and 54 male-sterile plants during the rainy season and fitted well to 3F:1S ratio \( (P = 0.59) \), but its corresponding BC₁ did not fit to 1F:1S ratio due to the excess of fertile plants (Table 3). However, the segregation pattern of this cross during the summer season had a good fit to both F₂ ratio of 3F:1S \( (P = 0.40) \) and BC₁ ratio of 1F:1S \( (P = 0.20) \). Such a segregation pattern in a different genetic background, represented by ICMA₄ 88004, indicated the dominant monogenic control of fertility restoration again. The ICMA₄ 88004 × IPC 804 cross had a good fit to the F₂ ratio of 3F:1S in rainy season \( (P = 0.51) \) and in summer season \( (P = 0.37) \) and also a good fit to the expected 1F:1S ratio in BC₁ in both the rainy and summer season \( (P = 0.07) \). The segregation pattern in F₂ of the ICMA₄ 88004 × IPC 1518 cross had a good fit in rainy season \( (P = 0.81) \), but its corresponding BC₁ did not fit to 1F:1S ratio due to the excess of fertile plants. In this cross, there was neither a good fit to 3F:1S in F₂ nor to 1F:1S ratio in BC₁ segregation in summer season due to the excess of fertile plants. The segregation pattern in this set of crosses revealed that out of the six cases of F₂s (three F₂s evaluated in two seasons), five cases had a good fit to 3F:1S ratio. However, out of the six cases of BC₁, only three cases had a good fit to 1F:1S ratio. In all these four cases (1 case of F₂ and three cases of BC₁) not conforming to single-gene segregation, there was an excess of fertile plants. Of these, two cases of deviation as observed in the rainy season could result from relatively lower temperatures and higher humidity that enhances the expression of modifiers for fertility restoration in pearl millet (RAI & HASH 1990). The effects of these modifiers could be inconsistent, depending on the genetic backgrounds of the segregating populations with the major genes for male sterility/fertility restoration present. Genetic studies in maize (Zea mays) (SINGH & LAUGHMAN 1972), sorghum (Sorghum bicolor) (TRIPATHI et al. 1985) and rapeseed (Brassica napus) (PAHWA et al. 2004) have shown a considerable effect of the genetic background and environments on the CMS inheritance. However, the excess of fertile plants as observed in the two remaining cases in the summer season could not be explained.

The segregation pattern observed in BC₁ F₂ progenies derived from three to seven BC₁ fertile plants of each of the six crosses showed all the progenies having a good fit to 3F:1S ratio, which is expected from the dominant monogenic control of fertility restoration (Table 4). The F₂s and BC₁s had a similar segregation pattern across both rainy and summer seasons in ten out of the 12 cases (six F₂s and six BC₁s evaluated in two

Table 3. Segregation for male-fertile (F) and male-sterile (S) plants in F₂ and BC₁ generations and the test of goodness of fit for hypothetical Mendelian ratios in crosses of ICMA₄ 88004 with the restorer parents IPC 511, IPC 804 and IPC 1518 in pearl millet, rainy and summer seasons 2010, Patancheru

<table>
<thead>
<tr>
<th>Cross</th>
<th>Generation</th>
<th>No. of plants observed</th>
<th>Expected ratio</th>
<th>( \chi^2 )</th>
<th>( P )</th>
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<td></td>
<td></td>
<td>F</td>
<td>S</td>
<td>F</td>
<td>S</td>
</tr>
<tr>
<td>ICMA₄ 88004 × IPC 511</td>
<td>F₂</td>
<td>176</td>
<td>54</td>
<td>3</td>
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<tr>
<td></td>
<td>BC₁</td>
<td>100</td>
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<td>1</td>
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<tr>
<td></td>
<td>F₂</td>
<td>122</td>
<td>47</td>
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<tr>
<td></td>
<td>BC₁</td>
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<td>ICMA₄ 88004 × IPC 804</td>
<td>F₂</td>
<td>214</td>
<td>65</td>
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<tr>
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<td>BC₁</td>
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<td>F₂</td>
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<tr>
<td>ICMA₄ 88004 × IPC 1518</td>
<td>F₂</td>
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<td>F₂</td>
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<td>BC₁</td>
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\( P \) – probability
Table 4. Segregation for male-fertile (F) and male-sterile (S) plants in BC$_1$F$_2$ progenies and the test of goodness of fit for 3F:1S segregation ratio in crosses of two A$_4$ CMS A-lines with three restorer parents in pearl millet, rainy season 2010, Patancheru

<table>
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<tr>
<th>Cross</th>
<th>Progenies</th>
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$P$ – probability
seasons) indicating that the season did not have much effect on fertility restoration of the \(A_4\) CMS system. This was also evidenced by the two remaining cases (BC\(_1\) of ICMA\(_8\), 88004 × IPC 511 in rainy season, and F\(_2\) of ICMA\(_8\), 88004 × IPC 1518 in summer season) where segregation distortion was observed in one case in either of both seasons. The overall segregation patterns of male sterile (S) and fertile (F) plants in populations derived from crosses between the A-lines (81A\(_4\) and ICMA\(_8\), 88004) and three diverse R-lines (IPC 511, IPC 804 and IPC 1518) provided the evidence of dominant single-gene segregation for fertility restoration with 3F:1S ratio in F\(_2\) and 1F:1S ratio in BC\(_1\) populations. Du et al. (1996) also reported a single dominant gene for fertility restoration of \(A_4\) cytoplasm in pearl millet, based on the study on a single cross evaluated in a single season. An earlier study found the dominant monogenic control of fertility restoration of the \(A_4\)-system of cytoplasmic-nuclear male sterility also in pearl millet (Yadav et al. 2010). The single-gene control of fertility restoration in the \(A_4\) CMS system, as revealed in this study, will help breeders to easily diversify the genetic base of restorers due to ease in the restorer breeding procedure, which in turn will help to diversify the cytoplasmic base of pearl millet hybrids.

**References**


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**Corresponding author:**

Shashi Kumar Gupta, Ph.D., International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), 502 324 Patancheru, Andhra Pradesh, India

e-mail: s.gupta@cgiar.org

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