

Genetics of cytoplasmic-nuclear male sterility and identification of molecular markers of fertility restorer genes in pearl millet (*pennisetum glaucum* (L.) R. Br)

Genetics of fertility restoration of diverse cytoplasmic-nuclear male sterility (CMS) systems in pearl millet was studied in segregating F₂, BC₁ and BC₂ populations of the crosses involving isonuclear A-lines of the five diverse CMS systems (A1, A4, Aegp, A5 and Av) in three diverse nuclear genetic backgrounds (81B, 5054B and ICMB 88004) and six pollen parents restoring the male fertility of hybrids based on any one, two or three male-sterile cytoplasm. Linkage between the fertility restorer genes of the A1 and A4 CMS systems, allelism among the fertility restorer genes of these CMS systems and molecular markers linked to fertility restorer genes of the A1 and A4 CMS systems were also studied. In a majority of crosses across the CMS systems, fertility restoration was governed by a trigenic inheritance mechanism represented either by dominant alleles of one basic gene and two duplicate-complimentary genes (F₂ ratio 45:19 and BC₁ ratio 1:1) or dominant alleles of any two of the three duplicate-complimentary genes (F₂ ratio 54:10 and BC₁ ratio 3:1). In few other crosses, different trigenic mechanisms with F₂ ratio of 57F:7S and 63F:1S and corresponding BC₁ ratio of 3F:1S and 7F:1S, respectively, were also observed

Although monogenic and digenic (F₂ ratio 15F:1S and 9F:7S and BC₁ ratio 3F:1S and 1F:3S, respectively) inheritance ratios were also observed in a few crosses, these resulted from the segregation of one or two genes out of the three involved in the trigenic inheritance. Segregation patterns of testcrosses from individual plants of F₂ and BC₁ populations derived from two B $\times$ R crosses were broadly supportive of the trigenic inheritance mechanism. Test of allelism studied from the fertility/sterility reaction of the three-way hybrids obtained by crossing A-lines with the F₁s of inter-crosses among three restorer lines (IPC 1518, IPC 511 and IPC 804) indicated the presence of same alleles of all the fertility restorer genes for the A1 CMS system, whereas different alleles for the A4 system. Joint segregation analysis revealed the presence of linkage between the fertility restorer genes of A1 and A4 CMS systems. A linkage map of 708.8 cM was constructed using 397 individuals and 36 molecular (SSR and RFLP) and morphological markers in the F₂ mapping population derived from the cross 81B $\times$ IPC 804. For the A1 CMS system, two QTL (Rf1a and Rf1b) and for the A4 system, three QTL (Rf4a, Rf4b and Rf4c) were identified with different unlinked genomic regions involved in the fertility restoration of these CMS systems. Based on the overall inheritance pattern observed, possible genotypes of the A-lines irrespective of CMS background were assigned as rf_a

rf_a
rf_b
rf_b
Rf_c Rf_c or rf_a rf_a rf_b rf_b rf_c rf_c and of the restorer lines as Rf_a Rf_a Rf_b Rf_b rf_c rf_c or Rf_a Rf_a Rf_b Rf_b Rf_c Rf_c (underscore to be replaced with the numbers `1`, `4` or `5` or alphabet `e` or `v` denoting the CMS systems). The information emanating from the study has implications in the breeding of maintainer and restorer lines of diverse CMS systems.

Table 6. Segregation for male-fertile (F) and male-sterile (S) plants in F₂ and BC₁ generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of three A1-system A-lines with the restorer parent IPC 1518, summer and rainy seasons 2003, ICRISAT- Patancheru

Parameter

No. of F₂ plants

No. of BC₁ (A $\times$ F₁) plants

Summer 2003

Rainy 2003

Summer 2003

Rainy 2003

Fertile

Sterile

Fertile

Sterile

Fertile

Sterile

Fertile

Sterile

81A1-P1 $\times$ IPC 1518-P3 (Hypothetical ratio in F2: 45F:19S; BC1: 1F:1S)

Observed

212

92

289

76

99

57

89

63

Expected

214

90

257

108

78

78

76

76

χ^2

0.02 (0.89)*

13.32 (< 0.01)

10.78 (< 0.01)

4.11 (0.04)

χ^2_p

6.49 (0.01)

14.57 (< 0.01)

χ^2_h

6.85 (0.01)

0.32 (0.57)

5054A1-P2 $\times$ IPC 1518-P1 (Hypothetical ratio in F2: 54F:10S; BC1: 3F:1S)

Observed

228

67

190

37

120

48

115

22

Expected

249

46

192

35

126

42

103

34

χ^2

10.71 (< 0.01)

0.04 (0.84)

0.96 (0.33)

5.37 (0.02)

χ^2_p

6.99 (0.01)

0.58 (0.45)

χ^2_h

3.76 (0.05)

5.75 (0.02)

88004A1-P3 $\times$ IPC 1518-P2 (Hypothetical ratio in F2: 45F:19S; BC1: 1F:1S)

Observed

197

129

245

74

93

77

64

56

Expected

229

97

224

95

85

85

60

60

χ^2

14.78 (< 0.01)

6.13 (0.01)

1.32 (0.25)

0.41 (0.52)

χ^2_p

0.90 (0.34)

1.82 (0.18)

χ^2_h

20.01 (< 0.01)

-0.09 (0.76)

*values in parenthesis are exact probability (P) values

χ^2_p is the Chi square value of the pooled data for both the seasons

χ^2_h is the heterogeneity Chi square value