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Research Article

Identification of potential maintainers and restorers using cytoplasmic male sterile lines in rice

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Abstract
Eight CMS lines were crossed as lines with 31 genotypes as 'testers' to get 248 hybrids. The 248 hybrids were subjected to pollen and spikelet fertility analysis. Among the 248 hybrids 168 hybrids were expressed as restorers 52 as PR, 28 as maintainers. Ten tester's viz., IR 62037, IR 72865, IR 68427, MDU5, TP1021, RR363-1, RR 347-1, RR 286-1, ACK 99017 and ASD06-08 were identified as restorers for all the eight CMS lines.

Key Words: CMS lines, restorers, maintainers, Rice.

Introduction

The use of cytoplasmic genetic male sterility system in developing hybrids in crops is possible only when effective restorers are identified. The CMS lines introduced from China are unstable to use as such in developing hybrid rice in India. Therefore, it is imperative to identify maintainers and restorers among the lines developed through conventional breeding procedures. Pollen (or) spikelet fertility or both have been used as an index to fix the restoration ability of the lines (Sutaryo, 1989). The present study was undertaken to find out the fertility restoration ability of the 31 testers on the eight CMS lines.

Materials and Methods

Eight cytoplasmic male sterile lines viz., IR 80559 A (L1), APMS 2 A(L2), IR 72081 A(L3), IR 75601 A(L4), IR 75596 A(L5), IR 80154 A(L6), CRMS 32 A(L7), IR 75608 A (L8) and 31 testers viz., IR 62124-83-3-2-1(T1), IR 62036-222-3-3-1-2(T2), IR 62037-93-1-3-1-1(T3), IR 63881-49-2-1-3-2(T4), IR 72865-94-3-3-2(T5), IR 62030-83-1-3-2(T6), IR 59673-93-2-3-3(T7), IR 68427-8-3-3-2(T8), IR 68926-61-2(T9), MDU 5(T10), ACK 99017(T11), AD 01259(T12), AD 01260(T13), TP 1021(T14), RR 363-1(T15), RR 361-3(T16), RR 354-1(T17), RR 347-1(T18), RR 348-6(T19), RR 286-1(T20), RR 166-645(T21), RR433-1(T22), RR 434-3(T23), ASD 06-1(T24), ASD 06-2(T25), ASD 06-3(T26), ASD 06-4(T27), ASD 06-5(T28), ASD 06-6(T29), ASD 06-7(T30) and ASD 06-8(T31) were raised in the LXT (8X31) mating design to get 248 hybrids during September 2007. Three stageared sowing were taken to synchronize the flowering.

Crossing was done by adopting clipping method. In the CGMS lines individual plants with complete pollen sterility was identified by observing the pollen grains under the microscope using one per cent Iodine potassium iodide stain. The spikelets were clipped off one third from the top without damaging the stigma, between 7.00 and 9.30 a.m. Immediately after clipping, the panicles were covered with butter paper covers. At the time of anthesis, panicles with fully opened spikelets were collected from the male parents and the pollen grains were dusted over the clipped panicles between 10.30 and 11.30 am. Crosses were effected between female and male parents in Line x Tester fashion and a total of 248 cross combinations were obtained. After 25 days, the matured panicles were harvested. The F1 generations of all the crosses were raised during April 2008 in a test cross nursery. Each entry was planted with a spacing of 20X20 cm with two replications. Identification of maintainers and restorers was carried out by observing pollen and spikelet fertility under bagged condition. Potential maintainers were identified as having >90% pollen sterility and <10% spikelet fertility and effective restorers as having <10% pollen sterility and >90% spikelet fertility.

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Results and discussion

The pollen fertility per cent of hybrids was varying from 0.92 (L5 x T 26) to 100% (L3 x T6). A very low magnitude of pollen and spikelet fertility was observed for hybrids (Table 1). The lines identified as effective maintainers can be further back crossed with their respective F1's to look for completely sterile back cross progenies so that these can be developed as new CMS lines.

In some cases, the same genotype behaved as a restorer for one CMS line and as a maintainer for the other CMS line. Tester IR62036 behaved as an effective restorer for CMS lines APMS 6A, IR80154A and IR75608 and was found to be partial restorer for other CMS lines. A Tester IR62037, IR72865, IR68427, MDU5 ACK99077, TP1021, RR363-1, RR347-1, RR286-1, and ASD06-8 were an effective restorer for all the right CMS lines. The tester ASD06-03 behaved as effective maintainer for APMS 2A and IR75596A were found to be effective restorers for other CMS lines.

The variations in behaviour of fertility restoration indicate that either the fertility-restoring genes are different or that their penetrance and expressivity varied with the genotypes of the parents or the modifiers of female background. This kind of the differential reaction of the same genotype in restoring the fertility of different CMS lines of same cytoplasmic source was reported by Gannamani (2001), Sao (2002), Hariprasanna et al. (2005) and Murugan and Ganesan (2006). This could be due to differential nuclear cytoplasmic interactions between the testers and CMS lines. The spikelet fertility of F1S ranged from 10.52 (L2 x T 26) to 96.82 (L3 x T20) (Table 2). The tester T4 exhibited partial sterility with L1 (13.42), but behaved as restoration with L2 (85.82).

None of the tester behaved as complete maintainer with the 8 CMS lines. The potential restorers and maintainers were identified and presented in table 3. The potential maintainers for all the CMS lines are being used in the back cross program to develop new CMS line. There were instances which the classification of tester based on the pollen fertility did not correlate with the classification based on the spikelet fertility. For example IR59673 with IR80559A and ADO1259 with AMPS2A and RR363-1 with IR72081-A were categorized as restorers by pollen fertility and as partial fertile by spikelet fertility analysis. Tester RR433-1 behaved as an effective maintainer for APMS2A and IR75596 was found to be an effective restorer for CMS lines L1, L4, L6, and L7. Such non-correlation between pollen fertility and spikelet fertility was reported by Murugan and Ganesan (2006) reported TKM6 identified as permanent restorer. A.N.Chi has been reported to be a restorer at Faizabad and maintainer at Coimbatore (DRR, 1997). Such a differential reaction of pollen parents at different locations may be attributed to genic x environmental interaction or due to some minor differences in genetic constitution of the parents maintained at different centres. The testers viz., T3, T5, T8, T10, T11, T14, T15, T18, T20 and T31 behaved complete restorers for all the eight cms lines. These testers could be utilized in the heterosis breeding after testing their combining ability and heterosis.

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Table 1. Pollen fertility per cent of F1 hybrid in test cross nursery

Lines/ Testers	L1	L2	L3	L4	L5	L6	L7	L8
T1	52.65 (P.R)	67.12(R)	36.48 (P.R)	72.86(R)	86.60(R)	98.83(R)	76.36(R)	52.43(P.R)
T2	45.05 (P.R)	82.76(R)	48.78(P.R)	56.42(P.R)	36.00(P.R)	98.47(R)	42.48(P.R)	76.00(R)
T3	93.43 (R)	90.91(R)	98.24(R)	70.88(R)	72.46(R)	85.34(R)	92.72(R)	68.86(R)
T4	0.83 (M)	93.17(R)	98.91(R)	96.73(R)	86.92(R)	36.82(P.R)	42.48(P.R)	0.82(M)
T5	85.56 (R)	64.82(R)	91.03(R)	97.74(R)	68.36(R)	73.64(R)	77.42(R)	72.36(R)
T6	97.19 (R)	86.24(R)	100.0(R)	93.39(R)	92.42(R)	14.80(P.M)	93.97(R)	36.43(P.R)
T7	84.25 (R)	89.47(R)	72.42(R)	91.47(R)	58.21(P.R)	92.56(R)	36.72(P.R)	90.86(R)
T8	67.27 (R)	76.42(R)	86.93(R)	82.00(R)	76.48(R)	78.00(R)	92.43(R)	78.48(R)
T9	49.47 (P.R)	88.16(R)	54.42(P.R)	86.84(R)	54.26(P.R)	42.14(P.R)	96.23(R)	68.56(R)
T10	69.29(R)	63.00(R)	100.0(R)	88.33(R)	84.62(R)	78.46(R)	82.28(R)	96.48(R)
T11	94.70(R)	94.44(R)	82.44(R)	80.39(R)	62.82(R)	82.52(R)	97.26(R)	92.82(R)
T12	97.32(R)	89.15(R)	85.86(R)	93.88(R)	97.10(R)	16.46(P.M)	34.72(P.R)	38.24(P.R)
T13	93.40(R)	92.46(R)	84.72(R)	94.89(R)	90.73(R)	42.52(P.R)	97.95(R)	18.48(P.M)
T14	95.74(R)	92.59(R)	100.0(R)	92.42(R)	94.86(R)	81.82(R)	78.57(R)	64.76(R)
T15	78.68(R)	72.46(R)	68.46(R)	94.04(R)	82.66(R)	68.00(R)	82.98(R)	72.38(R)
T16	58.00(P.R)	54.82(P.R)	42.82(P.R)	83.92(R)	12.66(P.M)	52.58(P.R)	46.76(P.R)	0.72(M)
T17	94.60(R)	65.66(R)	79.78(R)	90.02(R)	32.00(P.R)	76.38(R)	76.43(R)	28.76(P.R)
T18	70.59(R)	96.42(R)	75.46(R)	94.41(R)	72.58(R)	96.42(R)	72.78(R)	77.42(R)
T19	93.75(R)	43.91(P.R)	82.84(R)	46.76(P.R)	82.42(R)	48.63(P.R)	54.63(P.R)	56.86(P.R)
T20	88.89(R)	78.86(R)	92.00(R)	96.51(R)	68.58(R)	72.48(R)	97.75(R)	78.00(R)
T21	83.14(R)	84.07(R)	42.82(P.R)	88.02(R)	36.86(P.R)	86.73(R)	90.48(R)	84.28(R)
T22	98.35(R)	0.86(M)	36.46(P.R)	68.72(R)	20.96(P.R)	92.43(R)	72.86(R)	36.48(P.R)
T23	33.78(P.R)	72.46(R)	78.32(R)	56.05(P.R)	37.89(P.R)	68.62(R)	98.13(R)	96.34(R)
T24	33.89(P.R)	0.92(M)	0.62(M)	0.72(M)	20.87(P.R)	0.96(M)	16.78(P.M)	0.86(M)
T25	70.34(R)	58.42(P.R)	47.74(P.R)	48.46(P.R)	30.81(P.R)	0.94(M)	48.43(P.R)	13.82(P.M)
T26	92.62(R)	0.88(M)	85.38(R)	87.50(R)	0.00(M)	81.18(R)	86.74(R)	24.92(P.R)
T27	90.41(R)	18.26(P.M)	68.43(R)	18.42(P.M)	13.84(P.M)	56.11(P.R)	56.42(P.R)	18.76(P.M)
T28	42.76(P.R)	57.22(P.R)	72.82(R)	76.78(R)	95.45(R)	52.72(P.R)	68.73(R)	68.54(R)
T29	89.53(R)	72.56(R)	48.46(P.R)	97.28(R)	80.43(R)	88.56(R)	49.42(P.R)	76.48(R)
T30	72.76(R)	68.58(R)	0.76(M)	97.54(R)	80.98(R)	32.43(P.R)	38.76(P.R)	17.86(P.M)
T31	68.42(R)	93.22(R)	94.15(R)	72.82(R)	98.44(R)	74.00(R)	76.32(R)	92.74(R)

M : Maintainer PM : Partial maintainer PR : Partial restorer
R : Restorer

Table 2. Spikelet fertility per cent of F1 hybrid in test cross nursery

Lines/ Testers	L1	L2	L3	L4	L5	L6	L7	L8
T1	68.72 (PF)	63.25 (PF)	52.76(PF)	86.76(F)	89.58(F)	83.01(F)	84.86(F)	68.76(PF)
T2	65.19(PF)	86.07(F)	70.26(PF)	68.42(PF)	52.76(PF)	86.43(F)	58.32(PF)	86.32(F)
T3	88.58(F)	88.72(F)	88.98(F)	84.76(F)	82.76(F)	85.57(F)	85.68(F)	74.82(PF)
T4	13.42(PS)	85.82(F)	84.26(F)	86.53(F)	88.42(F)	52.76(PF)	66.76(PF)	20.72(PS)
T5	91.63(F)	86.96(F)	88.97(F)	85.56(F)	76.38(PF)	71.71(PF)	88.40(F)	80.92(F)
T6	84.61(F)	82.56(F)	92.35(F)	86.74(F)	88.92(F)	23.53(PS)	83.12(F)	48.12(PF)
T7	69.47(PF)	86.32(F)	84.72(F)	91.01(F)	66.72(PF)	88.66(F)	58.46(PF)	86.52(F)
T8	62.38(PF)	81.52(F)	89.17(F)	88.42(F)	82.76(F)	86.32(F)	90.48(F)	84.62(F)
T9	83.30(F)	89.29(F)	66.52(PF)	82.76(F)	68.46(PF)	56.78(PF)	94.86(F)	72.56(PF)
T10	84.15(F)	83.35(F)	88.20(F)	86.96(F)	82.58(F)	80.92(F)	88.32(F)	84.82(F)
T11	92.02(F)	90.14(F)	84.56(F)	82.40(F)	81.72(F)	88.76(F)	88.26(F)	88.46(F)
T12	90.65(F)	63.14(PF)	86.56(F)	88.15(F)	86.73(F)	28.42(PS)	62.76(PF)	60.76(PF)
T13	91.92(F)	90.60(F)	88.78(F)	86.40(F)	82.59(F)	62.76(PF)	94.49(F)	26.46(PS)
T14	87.21(F)	91.83(F)	87.15(F)	90.52(F)	86.70(F)	87.05(F)	78.30(PF)	48.76(PF)
T15	88.46(F)	82.48(F)	82.82(F)	85.37(F)	88.48(F)	86.25(F)	73.41(PF)	82.12(F)
T16	52.78(PF)	66.72(PF)	62.76(PF)	91.29(F)	22.76(PS)	68.36(PF)	68.72(PF)	18.26(PS)
T17	80.54(F)	80.60(F)	81.39(F)	86.73(F)	46.76(PF)	81.38(F)	81.86(F)	48.76(PF)
T18	87.73(F)	88.13(F)	82.42(F)	87.47(F)	80.58(F)	92.42(F)	88.42(F)	82.43(F)
T19	87.18(F)	56.86(PF)	88.76(F)	66.70(PF)	86.76(F)	56.72(PF)	62.84(PF)	68.72(PF)
T20	93.65(F)	80.76(F)	96.82(F)	87.82(F)	62.46(PF)	86.46(F)	88.52(F)	84.16(F)
T21	86.79(F)	82.40(F)	58.36(PF)	88.80(F)	56.92(PF)	88.82(F)	93.43(F)	90.12(F)
T22	87.40(F)	13.72(PS)	48.72(PF)	76.42(PF)	43.71(PF)	90.76(F)	81.72(F)	52.18(PF)
T23	86.54(F)	81.56(F)	80.76(F)	85.90(F)	72.46(PF)	72.86(PF)	90.99(F)	88.42(F)
T24	57.41(PF)	16.48(PS)	18.46(PS)	20.72(PS)	78.04(PF)	13.72(PS)	32.46(PF)	18.46(PS)
T25	54.40(PF)	62.76(PF)	53.58(PF)	58.48(F)	67.68(PF)	8.16(PS)	62.72(PF)	28.12(PS)
T26	91.41(F)	10.52(PS)	80.78(F)	90.14(F)	27.78(PS)	86.43(F)	88.48(F)	32.48(PF)
T27	91.24(F)	14.72(PS)	72.86(PF)	26.56(PS)	38.22(PF)	79.30(PF)	46.72(PF)	22.86(PS)
T28	56.46(PF)	82..85(F)	84.72(F)	80.96(F)	88.46(F)	68.72(PF)	76.42(PF)	72.14(PF)
T29	86.12(F)	63.61(PF)	56.72(PF)	84.98(F)	82.22(F)	82.76(F)	52.45(PF)	81.48(F)
T30	68.76(PF)	82.52(F)	18.72(PS)	82.22(F)	83.67(F)	48.30(PF)	42.87(PF)	26.72(PS)
T31	72.48(PF)	84.29(F)	91.22(F)	82.76(F)	86.39(F)	82.72(F)	82.42(F)	86.14(F)

S : Sterile PS : Partial sterile PF : Partial fertile
F : Fertile

Table 3. Potential restorers and maintainers for eight CMS lines

CMS lines	Restorers	Maintainers
IR 80559	IR 62037-93-1-3-1-1**,** IR 72865-94-3-3-2, IR 62030-83-1-3-2, IR 59673-93-2-3-3, IR 68427-8-3-3-2, MDU 5, ACK 99017, AD 01259, AD 01260, TP 1021, RR 363-1, RR 354-1, RR 347-1, RR 348-6, RR 286-1, RR 166-645, RR433-1, ASD 06-2, ASD 06-3, ASD 06-4, ASD 06-6,ASD 06-7, ASD 06-8.	IR 63881-49-2-1-3-2
APMS 2	IR 62124-83-3-2-1, IR 62036-222-3-3-1-2, IR 62037-93-1-3-1-1, IR 63881-49-2-1-3-2, IR 72865-94-3-3-2, IR 62030-83-1-3-2, IR 59673-93-2-3-3, IR 68427-8-3-3-2, IR 68926-61-2, MDU 5, ACK 99017, AD 01259, AD 01260, TP 1021, RR 363-1,RR 354-1, RR 347-1, RR 286-1, RR 166-645, RR 434-3, ASD 06-6 ASD 06-7, ASD 06-8.	RR433-1, ASD 06-1, ASD 06-3
IR 72081	IR 62037-93-1-3-1-1, IR 63881-49-2-1-3-2, IR 72865-94-3-3-2, IR 62030-83-1-3-2, IR 59673-93-2-3-3, IR 68427-8-3-3-2, MDU 5, ACK 99017, AD 01259, AD 01260, TP 1021, RR 363-1, RR 354-1, RR 347-1, RR 348-6, RR 286-1, RR 434-3, ASD 06-3, ASD 06-4, ASD 06-5, ASD 06-8	ASD 06-1, ASD 06-7
IR 75601	IR 62037-93-1-3-1-1, IR 62124-83-3-2-1, IR 63881-49-2-1-3-2, IR 72865-94-3-3-2, IR 62030-83-1-3-2, IR 59673-93-2-3-3, IR 68427-8-3-3-2, IR 68926-61-2, MDU 5, ACK 99017, AD 01259, AD 01260, TP 1021, RR 363-1, RR 361-3, RR 354-1, RR 347-1, RR 286-1, RR 166-645, RR433-1, ASD 06-3, ASD 06-5, ASD 06-6, ASD 06-7, ASD 06-8.	ASD 06-1
IR 75596	IR 62124-83-3-2-1, IR 62037-93-1-3-1-1, IR 63881-49-2-1-3-2, IR 72865-94-3-3-2, IR 62030-83-1-3-2, IR 68427-8-3-3-2, MDU 5, ACK 99017, AD 01259, AD 01260, TP 1021, RR 363-1, RR 347-1, RR 348-6, RR 286-1, ASD 06-5, ASD 06-6, ASD 06-7, ASD 06-8	ASD 06-3
IR 80154	IR 62124-83-3-2-1, IR 62036-222-3-3-1-2, IR 62037-93-1-3-1-1, IR 72865-94-3-3-2, IR 59673-93-2-3-3, IR 68427-8-3-3-2, MDU 5, ACK 99017, TP 1021, RR 363-1, RR 354-1, RR 434-3, RR 347-1, RR 286-1, RR 166-645, RR433-1, ASD 06-3, ASD 06-6, ASD 06-8.	ASD 06-1, ASD 06-2
CRMS 32	IR 62124-83-3-2-1, IR 62036-222-3-3-1-2, IR 62037-93-1-3-1-1, IR 72865-94-3-3-2, IR 62030-83-1-3-2, IR 68427-8-3-3-2, IR 68926-61-2, MDU 5, ACK 99017, AD 01260, TP 1021, RR 363-1, RR 354-1, RR 347-1, RR 286-1, RR 166-645, RR433-1, RR 434-3, ASD 06-3, ASD 06-6, ASD 06-8.	-----
IR 75608	IR 62036-222-3-3-1-2, IR 62037-93-1-3-1-1, IR 72865-94-3-3-2, IR 59673-93-2-3-3, IR 68427-8-3-3-2, IR 68926-61-2, MDU 5, ACK 99017, TP 1021, RR 363-1, RR 347-1, RR 286-1, RR 166-645, RR 434-3, ASD 06-5, ASD 06-6, ASD 06-8.	IR 63881-49-2-1-3-2, RR 361-3, ASD 06-1