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Identification and Characterization of Promising TGMS Mutants with Good Floral Traits in Rice

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The current investigation was aimed to develop mutants with respect to pollen / spikelet sterility and good floral traits for use in two-line breeding. Two TGMS lines *viz*., TS 6 and CBTS 0282 were subjected to mutagenesis with gamma rays (300 and 350 Gy) and Ethyl methane sulphonate (0.5 percent and 0.6 percent) for developing new TGMS lines with desirable floral traits. The highest mean level of gain of function for well exerted stigma over check (IR 58025A) were observed in CBTS 0282 - 0.5 per cent EMS treated 27-4-1-7-4 (124.7 percent), TS 6 - 0.6 per cent EMS 196-1-3-6-3 (122.21 percent), TS 6 - 0.6 per cent EMS 196-1-3-4-1 (120.97 percent) and CBTS 0282 - 0.5 per cent EMS 27-4-1-3-3 (120.94 percent). These mutant populations also showed significant gain of function for days to 50 percent flowering, pollen sterility percentage and spikelet sterility percentage. Hence identified new lines are very much useful for using as parental lines in future two line breeding programme.

Key words: TGMS lines, mutagenesis, mutants, EMS, pollen and spikelet sterility

Rice (*Oryza sativa* L.) being an ancient and important food crop is grown world wide. To meet the demand of increasing population and maintaining self-sufficiency in food improving the yield of cereal crops namely rice, wheat corn *etc*., is very essential (Yuan and Peng 2005).

Potential yield of the modern semi-dwarf rice varieties released after IR 8 are more or less the same and showing a yield plateau or ceiling. Thus, hybrid rice technology is most feasible and readily adaptable one to meet the global rice demand. China's success in commercial hybrid production clearly demonstrates that hybrid rice at present is the only practical tool for increasing global rice production. Many years of practice and experience has proved that three-line breeding utilizing a cytoplasmic genic male sterility system (CMS) is the effective way to develop rice hybrids and will continue to play an important role in heterosis breeding. But this system has some constraints such as plateauing yield in rice hybrids, dependency on a single CMS source (WA), restriction on choice of male parents due to problems associated with fertility restoration, complex seed production procedures and high seed cost (Yuan, 1997).

The two-line system of hybrid breeding utilizing environment-sensitive genic male sterility (EGMS) is considered as an alternative to overcome the problems associated with three-line breeding and to exceed the yield plateau of three line hybrids. For breeding two-line hybrids under tropical conditions, where day length differences are marginal, a temperature-sensitive genic male sterile (TGMS) system is considered more useful than the photo period- sensitive genic male sterile (PGMS) system (Virmani, 1997). Male sterility expression in a TGMS line is heritable but regulated by temperature. At certain temperatures occurring during panicle development stages, the male sterility is altered into partial to complete fertility. This characteristic of the TGMS system eliminates the need for a maintainer line for its multiplication. Also the system does not require a restorer line. Since the cytoplasm is not involved in the sterility expression, plant breeders can develop hybrids with diverse cytoplasmic backgrounds to reduce the risk of their potential genetic vulnerability. The seed yield in hybrid rice seed production depends on the out crossing potential of the parental lines. Two line breeding by exploitation of thermo- sensitive genic male sterile system is pursued with the objective of development of TGMS parental lines with desirable floral traits. Hence diversification of TGMS system through mutation breeding by using the TGMS lines TS 6 and CBTS 0282 is being attempted.

Materials and Methods

Mutation breeding has been accepted as an useful mean of adding valuable attributes to a variety. Plant breeders have used this tool for the improvement of some cultivated crop varieties. The current investigation was aimed to develop mutants with complete pollen sterility / spikelet sterility and desirable floral traits in TGMS lines for use in two-line breeding. Two TGMS lines *viz.,* TS6 and CBTS 0282 were subjected to mutagenesis with gamma rays (300 and 350 Gy) and Ethyl Methane Sulphonate

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(EMS) (0.5 percent and 0.6 percent concentration) for developing new temperature-sensitive genic male sterile (TGMS) lines. The seeds treated with gamma ray and EMS were raised in M_1 generation and seeds were collected and forwarded to M_2 generation as plant to progeny rows for screening the best TGMS lines with desirable floral traits. In M_2 generation sterile, uniform individual plants with good floral traits were selected and stubbles were transplanted at Hybrid Rice Evaluation Centre, Gudalur, a low temperature region (Fertility favouring environment) for getting pollen and spikelet fertility. During that season fertile seeds were collected and raised as M_3 in fertility limiting environment at (Coimbatore). The same procedure was carried out upto M_4 generation. M_5 generation were raised along with their parents (TS 6 and CBTS 0282) and one check (IR58025 A) in fertility limiting environment (Coimbatore) to study their stability of fertility / sterility behaviour and desirable floral traits. From the mutagenesis chemical mutagen treated populations produced desirable mutants. Hence these populations alone were forwarded to M_5 generation. The mutagenic effects in terms of mean percent increase or decrease over standard check was worked out and presented in Table 1.

Results and Discussion

In M_4generation 36 individual progenies/ population were selected and forwarded to M_5 generation (fertility limiting environment). The floral traits of the mutants were studied and the results were discussed hereunder. Days to 50 per cent flowering ranged from 81 days (27-4-1-5-2) to 99 days (27-4-7-2-2) for CBTS 0282-0.5 per cent EMS treated population. In case of TS 6 - 0.6 per cent EMS treated population, days to 50 per cent flowering ranged from 84 days (207-1-28-3-2) to 102 days (196-1-4-1-2). Days to 50 per cent flowering for IR 58025A (check), CBTS 0282 and TS 6 were 85 days, 96 days and 110 days respectively. Hence development of intermediate flowering group between check and control plants was very much possible. In general, Desirable floral trait with medium duration maturity is an important criterion for developing new TGMS lines. Here 50 per cent flowering was observed to range between 81 days to 111 days in the mutants.

The mean pollen sterility was ranged from 63.0 per cent (27-4-1-6-6 and 27-4-12-5-3) to 100 per cent (27-4-1-3-3, 27-4-1-7-2, 27-4-6-1-2, 27-4-7-2-2, 27-4-7-3-1 and 27-4-12-4-3) in CBTS 0282 mutants. In TS 6- 0.6 per cent EMS treated mutants a total of eight progenies were observed with complete pollen sterility. In some of the sterile plants, absence of pollen grains was also observed. This phenomenon directly reflects complete sterility of the spikelets. These results were confirmed with the work of Yadav *et al.* (2008) and Jagatpati, (2008).

The stigma exertion percentage of the TGMS line is a very important trait in the hybrid seed production programme. The single side stigma exertion ranged from 43.48 per cent (27–4-1-5-2) to 62.07 per cent (27–4-7-3-1) in the mutated population of CBTS 0282. In case of TS 6 populations, single side stigma exertion ranged from 32.56 per cent (196-1-4-4-1) to 59.41 per cent (196 -1-3-2 -2).

Double side stigma exertion ranged from 22.22 per cent (27 -4-1-2-1) to 35.59 per cent (27-4-1-4-2) in CBTS 0282 - 0.5 per cent EMS treated population. The range of 17.79 per cent (196 -1-2-3-5) to 44.72 per cent (196-1-4-1-2) double side stigma exertion was recorded in 0.6 per cent EMS treated TS 6 mutant populations.Total stigma exertion percentage was from 52.85 per cent (27-4-12-4-3) to 94.10 per cent (27-4-1-3-3) in 0.5 per cent EMS treated populations. In case of TS 6 genotype, total stigma exertion ranged from 52.62 per cent (196-1-4-4-1) to 95.70 per cent (196-1-4-1-2). Most of the TGMS lines had more number of single side stigma exertions than double side stigma exertion.The seed yield in hybrid rice seed production depends on the out crossing potential of the parental lines.The identified sterile plant stigmas were broader and lengthier than normal fertile lines. Hence selection of TGMS parental lines based on stigma exertion percentage especially panicle with double side stigma exertion is more beneficial than single side stigma exertion percentage for getting higher seed production.

Most of the selected mutated population showed highly significant positive for most of the characters studied. The well exerted stigma was observed in the genotype of TS 6-0.6 per cent EMS treated population of 196-1-4-1-2 (95.70 percent) followed by 196-1-3-6-3 (94.60 percent), 196-1-3-4-1 (94.11 percent) and in CBTS 0282 - 0.5 per cent EMS treated population of 27-4-1-3-3 (94.10 percent). Singh and Rang (1999) also observed significant positive heterosis for stigma exertion percentage (ranged from 35.8 percent to 151.3 percent).

Among the CBTS 0282 -0.5 % EMS treated mutants, six mutant progenies produced complete spikelet sterility (100 percent). In case of TS 6, eight mutant progenies produced complete sterility of spikelet's. Similar results were reported by Pham *et al*. (2004) and Ravneet *et al*. (2007). In many of the progenies spikelet fertility percentage was much higher than the pollen fertility percentage.This indicates presence of few viable pollen grains may ensure high number of spikelet fertility. Hence identification of only complete pollen sterile progenies will be useful for hybrid rice breeding programme. Variations for stigma colour also were seen in some of the mutants. Two types of stigma colour were observed (white and purple) in the mutated population. In most of the population, purple coloured stigma was observed. Stigma colour change also confirms the occurrence of mutagenesis in the concerned genotypes.

Table 1.Mean performance of identified TGMS mutant population for desirable floral traits

Entries	Stigma Exertion (%)		Total	Days to 50% flowering	Pollen sterility (%)	Spikelet sterility (%)	Stigma Colour
	Single Side	Double Side					
CBTS 0282 - 0.5% EMS							
27-4-1-1-2	49.18	24.59	73.78	86	85	43	W
27-4-1-2-1	47.22	22.22	69.44	90	79	53	P
27-4-1-3-3	61.83	32.26	94.10	95	100	100	P
27-4-1-4-2	49.18	35.59	84.77	88	69	45	P
27-4-1-5-2	43.48	26.51	69.78	81	78	63	W
27-4-1-6-6	44.41	32.45	76.86	86	63	52	P
27-4-1-7-2	43.59	28.33	71.94	85	100	100	P
27-4-1-8-2	57.38	28.69	86.06	84	91	46	P
27-4-1-9-5	48.80	30.14	78.94	90	95	65	P
27-4-6-1-2	46.39	29.89	76.28	84	100	100	P
27-4-6-2-7	51.96	31.37	83.34	89	99	61	P
27-4-7-1-1	49.73	23.36	73.10	83	98	60	P
27-4-7-2-2	59.09	31.36	90.46	99	100	100	P
27-4-7-3-1	62.07	30.17	92.24	94	100	100	P
27-4-12-3-1	44.83	33.62	78.46	86	78	52	W
27-4-12-4-3	60.29	32.41	52.85	87	100	100	P
27-4-12-5-3	60.00	34.55	53.28	86	63	48	W
TS 6 - 0.6% EMS							
196-1-2-2-2	41.00	17.99	59.00	85	69	36	P
196-1-2-3-5	59.41	33.66	93.08	98	100	100	P
196-1-3-2-2	46.67	42.50	89.17	96	100	100	P
196-1-3-3-1	50.82	43.29	94.11	98	100	100	P
196-1-3-4-1	55.22	34.78	90.00	96	74	67	P
196-1-3-5-2	54.23	40.36	94.60	100	100	100	P
196-1-3-6-3	54.13	39.45	93.58	99	89	68	P
196-1-3-7-6	55.03	38.46	93.50	101	68	54	P
196-1-3-8-1	50.98	44.72	95.70	102	100	100	P
196-1-4-1-2	48.59	35.03	83.62	96	77	61	P
196-1-4-2-1	56.09	33.60	89.70	87	73	51	P
196-1-4-3-1	32.56	19.83	52.62	100	74	60	P
196-1-4-4-1	53.20	36.94	89.94	93	100	100	P
207-1-27-1-3	40.00	20.00	60.00	88	71	60	W
207-1-28-1-1	51.34	36.13	87.47	97	100	100	W
207-1-28-2-2	43.17	24.46	67.64	84	76	59	P
207-1-28-3-2	53.96	32.34	86.30	92	100	100	P
207-1-32-3-2	35.12	26.90	62.02	85	100	100	P
IR 58025A(c)	24.64	23.36	48.00	96	100	100	W
CBTS0282	17.92	16.87	34.50	110	100	100	P
TS6	49.18	24.59	73.78	86	97	90	W

W- White stigma, P- Purple stigma

From the study chemical mutagenproduced more number of desirable mutants than physical mutagens. In CBTS 0282-0.5% EMS treated genotype produced more number of viable mutants than CBTS 0282-0.6 % EMS. In TS 6 - 0.6 % EMS treatment produced more desirable mutants than 0.5 % EMS treated population. While comparing both the genotypes, TS 6 - 0.6 per cent EMS produced more number of desirable floral mutants than CBTS 0282. From the study TS 6-0.6 per cent EMS,196-1-3-6-3,196-1-3-4-1, ,196-1-4-1-2,196-1-3-2-2 and CBTS 0282-0.5 per cent EMS, 27-4-6-1-2, 27-4-1-3-3, 27-4-7-3-1 and 27-4-7-2-2 populations were identified for complete pollen sterility / spikelet sterility with desirable floral traits. These lines can be used for further hybrid rice breeding programme. These identified lines offer scope for utilizing as female parental lines in two line hybrid development with high yield in rice.

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