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INDUCTION OF MUTATIONS AFFECTING FLORAL TRAITS IN RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Four improved *indica* varieties adapted to high altitude regions namely K 39-2, IET 11988, Himdhan and IET 11990 were treated with ethyl methane sulfonate, sodium azide and gamma rays with the objective of determining the most efficient mutagen and most responsive genotype for induction of mutations affecting predominantly reproductive parts and their functioning in rice. Frequency and spectrum of mutations affecting floral traits computed on M_1 plant progeny and M_2 population basis revealed the combination of the efficient sodium azide and the most responsive variety IET 11990 to be ideal for recovering highest number of selectively male sterile mutations. Among three kinds of male steriles viz., typical abortive, sporogenous and deformed male sex types recovered, critical study of abortive type helped identify several temperature sensitive genic male steriles, two of which are being improved for commercial exploitation in two- line hybrid breeding.

Key words : Rice, induced mutagenesis, floral traits, male sterility

Advantages of male sterility in commercial exploitation of hybrid vigour and population improvement have been prompting crop breeders all through to look for usable male steriles of both spontaneous and induced origin. Mutagen induced male sterility has been reported in as many 35 crop species. In most of the instances recovery of male steriles has, however, not been from objectively planned experiments. Studies by various workers [1-5] in rice, and in barley [6] are among very few exceptions to this trend. Even these reports provide little information on relative efficacy of various mutagens for induction of mutations affecting reproductive system in general and development and functioning of floral parts selectively interfering with male fertility. Keeping this basic information lacunae in view the present study was undertaken in rice with the specific objective of identifying most responding genotype(s) and efficient mutagen(s) for induction and recovery of mutations affecting selectively floral characteristics including male fertility.

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MATERIALS AND METHODS

Four improved *indica* varieties of rice adapted to high altitude regions namely K 39-2, IET 11988, Himdhan and IET 11990 were chosen. Genetically pure and fully matured seeds of uniform size of the varieties were selected in lots of 3000 for mutagen treatment.

One seed lot with moisture content at 12% was exposed to acute dose of 25 kR of Gamma rays using the Gamma Cell (Cobalt 60 source) facility at the Indian Agricultural Research Institute, New Delhi. Another presoaked in water for 12 hr were treated with freshly prepared 0.1 percent ethyl methane sulfonate (EMS) solution in phosphate buffer adjusted to pH 7.0 for 6.0 hr at room temperature (25° ± 1°C). In respect of sodium azide (SA) seeds presoaked in water for 4 hr were treated with 0.002M mutagen solution prepared in citrate phosphate buffer adjusted to pH 3 for 6 hr at room temperature. The seed lots were subjected to low temperature (10°C) pre-treatment for 2hr with EMS sodium azide solutions at respective concentrations with the objective of maintaining the rate of the mutagen reaction at minimum and achieving thereby an equilibrium concentration of the mutagen inside the seed before the actual mutagen treatment. Seed lots presoaked in water and treated with respective buffer solutions for same period under identical conditions were kept as controls. After completion of the treatments, the seed lots were washed thoroughly in running water for 2 hr, so as to leach out the residual cellular mutagen from seeds.

The seeds thus treated with the physical and chemical mutagens were sown carefully in well prepared shallow pans to raise M_1 generation. Seedlings were transplanted after 25 days at one per hill adopting a spacing of 7 cm × 15 cm. Close planting and minimal fertilizer application were adopted to restrict secondary-tertiary tillering. Three first formed panicles from each of the M_1 plants were harvested and bulked as single plant produce. Seeds harvested from M_1 plants were sown to raise M_2 generation. 30 day old seedlings were transplanted at one seedling per hill adopting a spacing of 15 cm × 15 cm. The major objective of the study being for recovering mutations affecting sex expression, observations in the M_2 were confined to floral characteristics, which included structural and functional deformation, of reproductive parts. Suspected completely sterile and partially sterile plants were examined by physically opening the spikelets during anthesis. Those that had shrivelled, reduced, light to whitish yellow coloured and arrow-shaped anthers were taken as steriles, but after examination of their I-KI stained pollen under microscope.

Frequency of mutations was computed treatmentwise on M_1 plant progeny basis as percentage of M_1 plant progenies throwing mutations and on M_2 population

basis as percentage of mutations recovered. Mutation spectrum was computed as proportion (%) of various kinds of mutants to the total number of mutants recovered. Completely male sterile plants were crossed with the fertile parent variety to study the nature of genetic sterility in the F_1 and F_2 while maintaining them through cooler months (November) to watch for pollen/spikelet fertility in later-formed tillers. Also, stubbles of the sterile plants were raised under controlled glasshouse conditions for their reversible sterility/fertility behaviour with change of temperature regime to determine if any of them were temperature-influenced male steriles.

RESULTS AND DISCUSSION

Reports of successful recovery in mutagen treated populations of genetic male steriles [1, 3], cytoplasmic-genetic male steriles [7] and environment sensitive genic male steriles [4, 5] in rice prompted the present study primarily for exploring the possibilities of inducing temperature sensitive genic male steriles for developing two-line hybrid technology suited to tropical India.

The study was made with the specific objective of identifying appropriate genotype(s) that would respond to mutagen treatment and potent mutagen that would predominantly induce mutations affecting floral traits in general and selectively pollen fertility in particular. M_2 populations of the four varieties treated with gamma rays and two chemical mutagens viz. EMS and Sodium azide were screened under natural field conditions during wet (*Kharif*) season. The frequency of mutation computed on M_1 plant progeny and M_2 population basis reveals sodium azide to be the most efficient, the values being 29.77 and 2.99 per cent. Inducing less than one half of it gamma rays and EMS were on par (Table 1).

Table 1. Frequency of mutations affecting floral characteristics in M_2 generation of the variety Himdhan treated with gamma rays, EMS and sodium azide

Mutagens with dosage	Number of M_1 progeny rows studied	Number of M_2 plants studied	Frequency of mutations affecting floral characteristics	
			M_1 plant progeny basis	M_2 population basis
Gamma rays (25 kR)	72	3600	16.67	1.22
EMS (0.1%)	186	9300	16.67	2.22
Sodium azide (0.002M)	215	10750	29.77	2.99

Comparatively higher efficiency of sodium azide to induce mutations affecting floral traits is in conformity with the earlier reports on its efficiency to induce male sterile mutations in rice [7].

The spectrum of mutations broadly included steriles, multicarpelled, ovaryless, stamenless, abnormal glumed and non- flowering. Among the three mutagens, sodium azide has been found to induce all the floral variants closely followed by EMS with the spectrum remaining narrow in the gamma ray treated population (Table 2). As for mutagen specificity, sodium azide appears to affect male fertility the most, the frequency of completely sterile mutant being highest. As compared to the physical mutagen which induced predominantly mutations affecting glume shape/size, the chemical mutagens appear to induce largely point mutations as evident from the recovery of stamenfree, ovaryless and non-flowering mutants exclusively in EMS and sodium azide treatments. Besides the practical value of selective male sterile mutants those affecting various floral traits would form excellent experimental material for under standing the developmental genetics of reproductive parts. Also, induction of meiotic mutations, which is a possibility by this approach would be of value in understanding the genetic control of various stages of megasporogenesis leading to the development of apomicts in crops like rice as visualized by Kitada *et al.* [8].

Table 2. Spectrum of mutations affecting floral characteristics in M_2 generation of the variety Himdhan treated with gamma rays, EMS and sodium azide

Mutagen with dosage	Floral mutant traits (%)							
	CS	PS	MC	OL	SL	RS	NF	AG
Gamma rays (25 kR)	13.6	59.0	-	-	-	4.55	-	22.7
EMS (0.1%)	18.9	66.0	6.31	0.97	0.49	1.90	-	5.30
Sodium azide (0.002M)	32.7	48.2	1.25	0.31	0.62	1.56	0.62	14.3

CS : Complete sterile SL : Stamenless PS : Partial sterile RS : Reduced stamen number
MC : Multiple carpellary NF : Non flowering OL : Ovaryless AG : Abnormal glumes

As for the test material, varietal choice was restricted to varieties adapted to high altitude conditions in Himachal Pradesh and Jammu & Kashmir on the assumed high probability of recovering physiologically balanced mutations at desired temperature range of fertility transformation, the primary objective of the study being induction of temperature sensitive genic male sterle mutations. Like efficiency varying with the mutagen, genotypic response to any given mutagen has also been found to vary with the variety. Among the four varieties treated with sodium azide,

IET 11990 closely followed by Himdhan appear to be most responsive as measured by the frequency and spectrum of mutations computed on the basis of M_1 plant progeny and M_2 population. (Table 3 & 4).

Table 3. Frequency of mutations affecting floral characteristics in the M_2 generation of sodium azide treated material

Variety	Number of M_1 progeny rows studied	Number of M_2 plants studied	Frequency of mutations affecting floral characteristics	
			M1 plant progeny basis	M2 population basis
K 39-2	65	3250	13.85	0.98
IET 11988	70	3500	29.77	1.03
Himdhan	215	10750	29.77	2.99
IE 11990	280	14000	30.36	4.69

Generation of a desired class of mutations is not only dependent on the efficient mutagen and most responsive variety but equally so on the interaction of both. In the induction of floral trait related mutations, in rice, it appears that the combination of sodium azide and any sensitive variety like IET 11990 or Himdhan would prove highly rewarding. It is quite likely that sensitive varieties from other agro- ecologies might throw the male sterile mutants of different kind.

Table 4. Spectrum of mutations affecting floral characteristics in M_2 generation of sodium azide treated material

Variety	Floral mutant traits (%)							
	CS	PS	MC	OL	SL	RS	NF	AG
K 39-2	31.25	46.44	-	-	-	3.12	-	18.75
IET 11988	30.56	44.44	-	-	-	2.78	-	22.22
Himdhan	32.71	48.29	1.25	0.31	0.62	1.56	0.62	14.33
IET 11990	38.05	35.77	0.46	0.76	1.06	1.37	1.98	25.72

CS : Complete sterile PS : Partial Sterile SL : Stamenless RS : Reduced stamen number
MC : Multiple carpellary OL : Ovaryless NF : Non flowering AG : Abnormal glumes

The male steriles are known to be of different kinds viz., typical abortive, sporogenous (pollenless) and deformed male sex types. Study of the completely

sterile mutants, especially of the typical abortive type to know, if they are simply inherited genetic male steriles across temperature regimes and/or temperature sensitive genic male steriles has revealed none to be of typical genetic male sterile. Interestingly, however, fifteen of them induced by sodium azide, one by EMS to be of temperature sensitive. Among them F43 (SA2) and F61 have been identified to be most promising temperature sensitive genic male steriles with their critical fertility and critical sterility points in the most desired range. The mutant genes are now being transferred to productive agronomic backgrounds for development of commercially viable two-line hybrids.

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