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Full Length Research Paper

Ethyle methane sulphonate (EMS) induced mutagenic attempts to create genetic variability in Basmati rice

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Chemical mutagens have long been used to create genetic variability in crop plants for breeding research and genetic studies. In rice, more than 500 varieties have been released through induced mutations. Ethyl methane sulphonate (EMS) is the most commonly used chemical mutagen in plants. EMS normally induces Gas chromatography GC to AT transitions in the genome and thus resulting protein in the mutant plant has different functions than normal. The present study was carried out to investigate the mutagenic effects of different concentrations of EMS on germination and yield parameters of two basmati rice cultivars (Super basmati and Basmati 370). The seeds were subjected to different treatment levels of EMS. The treated and untreated plants were observed under different agronomic parameters. EMS was quite effective in inducing genetic variability in Basmati rice. The results revealed significant difference among all the traits studied. The efficiency of EMS was found to depend upon its concentration and it was higher at lower concentration in both genotypes. The study further revealed that the use of EMS is an effective approach for creating new rice germplasm.

Key words: Rice (*Oryza sativa* L.), induced mutations, mutation breeding, EMS.

INTRODUCTION

Basmati rices fetch higher price in international markets due to pleasant aroma and good quality of kernel. The demand of aromatic varieties increased significantly in the recent years as a result of food diversification. Pakistan is famous for producing and exporting long and extra long grain Basmati rice. As an important export item of the country, it accounts for 5.9% of value added in agriculture and 1.3% in gross domestic product (GDP) (Economic survey 2009 to 2010). Rice industry is an important source of employment for thousands of heads in the country. Therefore, improving the productivity of rice would contribute to hunger eradication, poverty alleviation, national food security and economic development of the country. Successful plant breeding depends on genetic variation in useful traits. Induced mutations (chemical and physical) are effective tools in obtaining new cultivars and broadening the genetic base of crops. About 70% mutant varieties were released directly as mutants while remaining 30% were developed through cross breeding programme where mutants served as a source of desirable alleles (Maluszynski et al., 2000). To date, 2,428 crop varieties have been released through mutagenesis; among them 501 are rice varieties.

Mutations produce raw materials for genetic improvement of crops (Adamu and Aliyu, 2007). Artificially induced mutations have greatly advanced the understanding of genetics of crop plants. It provides the possibility of inducing desired characters that either cannot be found in nature or have been lost during evolu-tion (Ahloowalia et al., 2004; Maluszynski and Szarejko, 2005). The use of induced mutations in model organisms has emergent interest to study the functional genomics (Liu et al., 1999; Nadeau and Frankel, 2000). Production of mutants through chemical or irradiation mutagenesis is relatively economical. Any genotype can be mutagenized and the distribution of mutations is usually random in the genome. EMS is one of the most frequently used alkylating agent for chemical mutagenesis in plants due to its potency and ease with which it can be used. EMS is

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Table 1. Chemical composition, physiochemical and cooking characteristics of Super Basmati and Basmati- 370.

Variety	Shape	Amylose content (%)	Gelatinisation temperature (°C)	Gel consistency
Super Basmati	Extra long cylinder	24.80	66	64± 0.2
Basmati- 370		23.50	65	61 ± 0.3

more effective than physical mutagens (Dhanayanth and Reddy, 2000; Bhat et al., 2005). It can proficiently induce chemical modification of nucleotides, which results in various point mutations such as nonsense, missense and silent mutations (McCallum et al., 2000a, 2000b).

It is mainly used to produce GC to AT transition (Rao, 1977; Koornneef et al., 1982). An important advantage of using EMS as a source of mutagenesis is that a substantial body of literature has accumulated that confirms its utility in forward genetic screens in a variety of organisms. Targeting induced local lesions in genome (TILLING) combines high density of point mutations provided by traditional chemical mutagens like EMS with rapid mutational screening to discover the induced mutations (McCallum et al., 2000). EMS results stable point mutations and thus produces an allelic series of truncation and missense changes that can provide a range of phenotypes. The present investigation was therefore performed to optimize and reduce the response of two basmati cultivars on different doses of EMS (a chemical mutagen). The progress in this experiment will greatly help us for the discovery of single-nucleotide polymorphism (SNPs) and induced mutations at advance level.

MATERIALS AND METHODS

Most widely grown basmati rice cultivars of Pakistan, Super basmati and Basmati-370 were used in this study. The two cultivars have good grain yield potential but are susceptible to most insect pests. Super basmati is a premier variety of Pakistan with long cylindrical grain, famous for its aroma and nuanced flavor. Basmati 370 is another aromatic cultivar of the country with elite cooking characters. The grain quality characteristics of both varieties are described in Table 1.

Reasons why these varieties are highly priced

Translucent appearance, uniform cylindrical shape, pleasant fragrance upon cooking, sweet taste, soft texture, freshness retains for more than 24 h, excellent palatibility, tenderness >80%, flakiness > 80%, no stickness or cohesiveness, excellent separatibility, elongation index range 1.02 to 1.35 with 0 to 2% bursing upon cooking and 0 to 4% curviness.

Mutagenesis

The experiment was conducted at National Institute for Biotechnology and Genetic Engineering (NIBGE) Faisalabad, Pakistan. For current study, the 4000 dry seeds of each genotype were presoaked in distilled water for 16 h at room temperature (24 to 28°C). The presoaked seeds were divided into two equal parts and one part was taken as control and the second half were treated with different concentrations of aqueous EMS (Sigma, USA) to produce mutants. For EMS mutagenesis, seeds were put in falcon tubes containing different concentrations of EMS solutions (0.0, 0.5, 1.0, 1.5 and 2.0%), for 6 h in the laboratory conditions with intermittent shaking to maintain uniformity. These doses were chosen because preceding germination assays marked that these doses may be suitable for mutation breeding. The seeds were then thoroughly washed under running tap water for 6 h to leach out the residual chemical and then dried. The treated seeds were divided into three groups (Petri plates, tubs and field).

In petri plates containing two layers of moist filter paper, seeds of both genotypes were sown in three replications against each concentration in lab conditions. The recording of data for germination % was started after two days of shifting the seeds into the petri plates. Then 100 seeds of each genotype against different concentrations of EMS were sown in the field to estimate the germination % in field conditions. Thirty days old nursery was transplanted to the field under randomized complete block design. The line to line and plant to plant distance was maintained 4.5 inch. No fertilizer was applied throughout the entire crop tenure. At maturity, data were recorded for the following parameters, plant height, productive tillers/plant, panicle length, no of filled grains/panicle, no of unfilled grains/panicle, total no of grains/panicle, panicle fertility and yield/plant. The methodology given by Steel et al. (1997) was used for statistical analysis to construct ANOVA to compare the differences of two genotypes at different doses of EMS. The germination % of both genotypes was recorded after six days of sowing the seeds in the field.

RESULTS AND DISCUSSION

The results from graph indicate that the germination % decreases with the increasing dose of EMS. After 6 days of sowing, Basmati-370 showed 96% germination at 0.0% EMS while at 0.5% EMS, it showed 87% germinat-ion. Similarly at the doses of 1.00 and 1.5%, basmati-370 showed 68 and 7% germination, respectively. No germination was noticed at 2.00% EMS for basmati-370. In case of Super basmati, germination % was 95% at 0.00% EMS and 88% at 0.5%. Similarly at the doses of 1.0 and 1.5%, Super basmati showed 60 and 16% germination %, respectively. At the dose of 2.00%, 10% germination% was noticed only in super basmati. The results of lab and field conditions for both genotypes were significant. Basmati-370 showed 100% germination at control and 0.5% EMS. At EMS dose of 1.0 and 1.5%, it showed 94 and 55% germination%, respectively. Figure 1 revealed that at the dose of 2.0%, 13% germination was recorded which is 13% more than filed conditions where no germination was observed. In case of super basmati, 100, 100, 80, 30 and 3% germination recorded at EMS

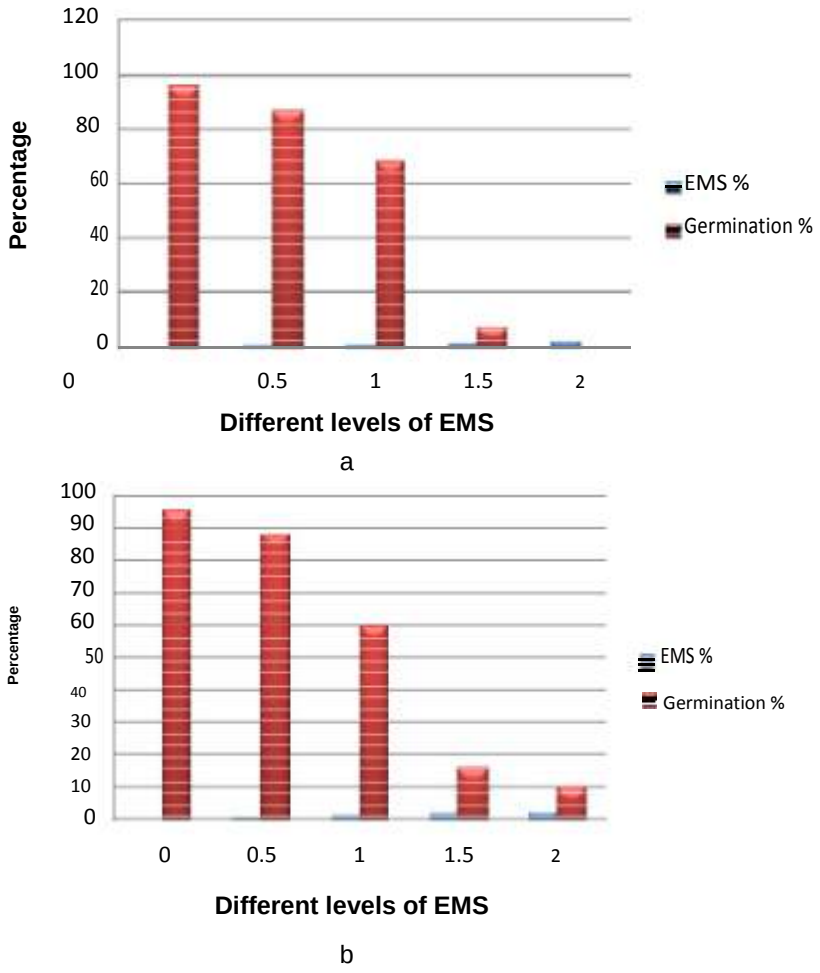

Figure 1. Germination % of Basmati-370 (a) and Super basmati (b) at different doses of EMS in lab.

concentration of 0.00, 0.5, 1.0, 1.5 and 2.0%, respectively (Figure 2). The results from both cultivars revealed that germination % was decreased with increasing concentration of EMS.

DISCUSSION

The results presented above clearly revealed that the performance of all the yield traits decreased with increasing dose of EMS. These mutants indicate that EMS can be used for the improvement of rice crop. Table 2 shows the analysis of variance for comparisons of means. These types of mutants were also reported earlier (Alcantara et al., 1996). The efficiency of a mutagenic agent is of complex nature and is mainly influenced by pH and temperature during treatment especially in case of nitroso amides (Veleminsky and Gichner, 1970). Some sterile mutants were also observed. Efficiency of a mutagen at lower concentration appears mainly due to the fact that injury, lethality and sterility increases with an increase in the mutagen concentration than actual mutations (Kharkwal, 1998; Cheema and Atta, 2003). Table 3 indicates the least significant (LSD) test of all the yield parameters studied at $p<0.05$. The variation in biological parameters viz., plant height and no of tillers may be attributed to a drop in auxin level (Gordon and Webber, 1955), chromosomal abrasions or due to decline of assimilation mechanism (Quastler and Baer, 1950). On the basis of lethality, the highest mutagenic efficiency was recorded at 2.0% EMS (highest dose).

Conclusions

The efficiency of EMS was higher at lower concentration. Results suggest that using a dose 0.5 to 1.0% of EMS for 6 h can induce mutations in rice. The mutant plants generated in this study would be used for linkage and

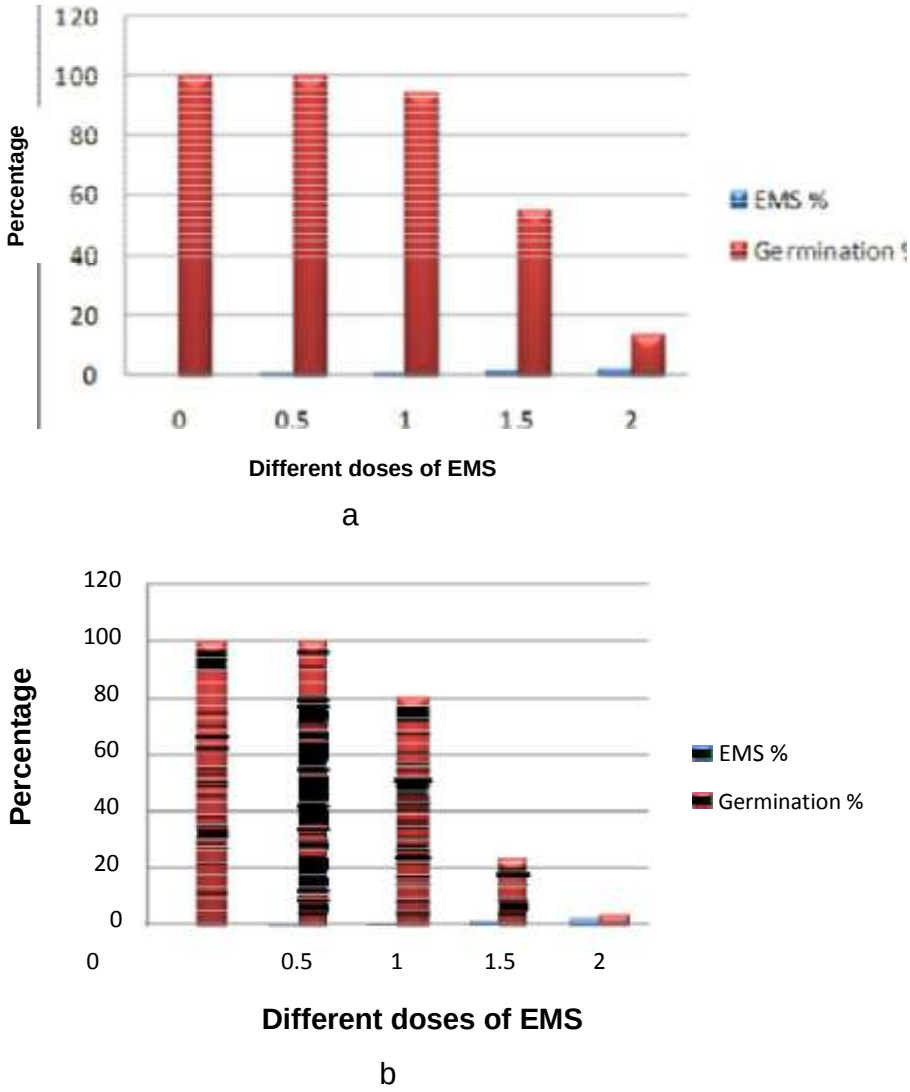

Figure 2. The germination % of Basmati-370 (a) and Super Basmati (b) in field conditions.

Table 2. Analysis of variance (ANOVA) table for comparison of means.

		Height	Tiller/plt	Pn length	No of grains/pn	Pn fertility	Yield/plant
Replication	2	45	32.01*	0.49	334.5	13.66	21.596
Treatment	4	124***	8.29	0.69	165.4	133.68*	24.981
Varities	1	13367***	66.19*	60.12***	1950.0*	115.21	2.089
Treatment* variety	4	136***	24.36	0.88	121.7	108.77*	19.645
Error	18						

Where *p<0.5, **p<0.01, ***p<0.001.

mapping studies of rice under different yield and grain quality parameters. These mutant plants could also be used as genetic markers. Thus mutation induction is a useful conventional breeding tool for developing superior cultivars. However with the development of novel approaches such as TILLING, a breeder could be able to identify useful mutations that might otherwise be ignored during the selection process.

Table 3. Least significant difference test (LSD) p< 0.05, where T1=0.0, T2=0.5, T3=1.0, T4=1.5 and T5=2.0%.

EMS Treatment	Height	Tiller/plt	Pn length	No. of grains/pn	Pn fertility	Yield/plant
B.370T1	97.5714^{a}	13.04762^{a}	23.5714^{a}	78.2146^{a}	78.2146^{a}	11.09524^{a}
B.370T2	91.7619^{bc}	6.85714^{a}	23.4286^{a}	94^{ab}	78.6969^{a}	7.2381^{a}
B.370T3	90.375^{c}	8.58333^{ab}	23.0417^{b}	81.8958^{a}	80.3886^{a}	9.91667^{b}
B.370T4	95.9524^{ab}	14.09524^{a}	23.7619^{c}	86.5714^{abc}	77.9812^{a}	7.19048^{a}
B.370T5	93.2222^{abc}	11.33333^{b}	24.3333^{ab}	87.1481^{a}	82.3094^{a}	12.55556^{a}
S.B T1	145.452^{a}	7.39683^{a}	27.4286^{a}	110.2222^{ab}	91.8604^{a}	13.63492^{ab}
S.B T2	142^{ab}	7.80952^{c}	26.1429^{a}	102.1905^{c}	87.3984^{a}	10.66667^{a}
S.B T3	138.571^{b}	9.85714^{a}	26.3333^{b}	104.3333^{a}	81.8901^{a}	12.33333^{a}
S.B T4	124.056^{a}	6.22222^{b}	26.1667^{a}	93.1111^{a}	69.0127^{b}	6.88889^{a}
S.B T5	129.889^{c}	7.77778^{a}	26.2222^{a}	114.6667^{a}	87.0259^{a}	7.11111^{b}

Means that were significantly different based on LSD test are shown with different letters (a,b,c,d). The values in the same column with different lower case letters indicate significant differences at p < 0.05

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