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Induction genetic variability using ems and its molecular analysis using RAPD, ISSR and SSR markers in cotton

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Abstract
The objective of the present work includes induction of genetic variability by chemical mutagen ethyl methane sulfonate and assessment variation in genomic DNA through RAPD, ISSR and SSR markers caused due to mutagenic effect of EMS in cotton. The two varieties of cotton *viz*., *Gossypium hirsutum* (GSHV-01/1338) and *Gossypium arboreum* (G.27) were subjected to different EMS concentration (0.75%, 1.5%, and 2.25%) treatment. Non treated plants were served as control. EMS @ 2.25% concentration found to be lethal in G.27. Observation indicated that as the concentration of EMS was increased, germination percentage, root length and shoot length was decreased. Further, biological effects of EMS on the total genomic DNA of the treated plants studied through RAPD, ISSR and SSR markers indicated, induced variation resulted in genetic distance to an extent of 2 per cent in G.27 and 1 per cent in GSHV (01/1338) in comparison to non-treated (control) varieties

Keywords: Cotton, genetic variability, markers, EMS

Introduction
Cotton is one of the most important commercial crop and is popularly known as "White Gold". In India, all the four cultivated cotton species *viz*., *Gossypium arboreum*, *G. herbaceum*, *G. hirsutum* and *G. barbadense* are grown on a commercial scale. These commercially grown cotton varieties have limited genetic diversity (Rahman *et al.*, 2002) [6]. Several decades of intensive breeding for better and improved cotton fibre traits; and evolutionary bottlenecks have narrowed the gene pool lead to reduced the genetic variation in modern cultivated cotton. Mutagenesis has been shown to be an effective tool to create a wide range of phenotypic variation in both diploid and tetraploid *Gossypium* populations (Auld *et al.*, 2000) [2]. Thus, creation new mutants through chemical mutagens is an alternative strategy to increase genetic variability. Ethyl methane sulphonate (EMS), a chemical mutagen reacts with DNA that leads to base-pair insertion or deletions change in the nucleotide sequence, thus resulting in point mutation. A majority of the changes (70–99%) in EMS mutated populations are GC to AT basepair transitions (Till *et al.*, 2007) [3]. Ali *et al.*, (2012) [1] studied EMS induced mutagenesis in Malaysian Rice (cv. MR219) for lethal dose determination. Seeds were treated with EMS at concentration of 0.25%, 0.75%, 1%.1.25%,1.5% and 2%. As the concentration of applied EMS increased, per cent germination, seedling height, root length and emergence under field conditions, decreased as compared to the control in M1 generation. Morphological features are indicative of the genetic potential but are represented by only a few loci and are also influenced by environmental conditions. Thus, estimation of genetic variation at molecular level is crucial. Presently, many kinds of DNA based molecular markers such as RAPD (Random Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeat), SSR (Simple Sequence Repeat), RFLP (Restriction Fragment Length Polymorphism) and AFLP (Amplified Fragment Length Polymorphism) are implemented to detect variation at the DNA level. Anil *et al.*, (2012) [5] studied RAPD analysis of EMS mutagenised mulberry genotype RFS135. The active bud sprouts of the mulberry genotype RFS135 was treated with EMS (0.1% and 0.3%) intermittently for the duration of twelve hours. The biological effects of EMS on the total genomic DNA of the treated plants were studied through RAPD technique. The M1V2 variant clones obtained from the plants treated with 0.1% and 0.3% concentrations of EMS revealed polymorphs of significantly variable size of different base pairs for two random primers used (OPW-04/05), thus indicating probable changes in the molecular characters of the total genomic DNA. Mutants screened at 0.1% and 0.3% showed higher levels of genetic variation and more unique alleles compared to the control due to EMS induced mutation. Thus, present

investigation was carried out to induce genetic variability in cotton using chemical mutagen, EMS and assessment of genetic variability using DNA based markers.

Methodology

Approximately 400 Seeds of two cotton varieties GSHV-01/1338 (*Gossypium hirsutum*) and G.27 (*Gossypium arboreum*) were obtained from Main Cotton Research Station, Navsari Agricultural University, Surat. Cotton seeds were delinted with concentrated sulfuric acid (100 ml /kg) then washed thoroughly in running tap water (Wu *et al.*, 2005) [9] and allowed to dry. The seeds of both the varieties were treated by soaking in three different concentrations of EMS (0.75%, 1.5% and 2.25%) for 8 hr at room temperature 28±2° C in a incubator shaker at 100 rpm, which provided uniform treatment to the seeds. For control reaction, plain distilled water was used. Treated seeds were allowed to germinate on sterile petriplates. Further, properly germinated seeds in petri plates were transferred to test tubes containing Hoagland solution in such a way that root portion were in contact with the Hoagland solution (Draper *et al.;* 1988) [4]. Test tubes were covered with cap and kept in growth room at 25±2° C to 16 hr photoperiod and seedlings were allowed to grow. Five seedlings were selected at random from control and treated seeds from both the varieties and their per cent germination, root length and shoot length were measured after 14 and 21 days.

Molecular diversity study

Genomic DNA was isolated from young fully expanded leaves of control and EMS treated plants both varieties by the CTAB method with minor modification (Saghai Maroof *et al.*, 1984) [7]. DNA was quantified according to Sambrook *et al.*, (1982) [8] method using UV visible spectrophotometer. Further, genomic DNA of both control and treated samples was subjected to polymerase chain reaction using twenty five RAPD oligonucleotide primers (GeNei), 19 ISSR and a set of 11 SSR primer pairs belonging to JESPR Series synthesized by (Eurofins Genomics India Pvt Ltd). Further, amplified DNA fragments of RAPD and ISSR were separated on 1.2 % agarose gels; and SSR products on 2% agarose gel stained with ethidium bromide. RAPD, ISSR and SSR profiles generated were computed using a NTSYS-pc analytical package. Bands were scored as 1 (present) or 0 (absent). Similarity coefficient was calculated using the Jaccard index and a cluster analysis was performed by Unweighted Pair Group Method Arithmetic Average (UPGMA), using the NTSYS-pc analytical software.

Result and Discussion

Effect of EMS on seed germination

Cotton seeds of both the varieties were germinated on moist filter paper and observation on seed germination were recorded at 7 and 14 days. As compared to the control, the germination % was lower in EMS treated in both cotton varieties as revealed in the Figure 1(a) and 1(b). Difference in germination percentage in the control and 0.75% EMS treated were relatively small between 7 days and 14 days incubation period in both the varieties of cotton. Whereas, at 2.25% EMS concentration minimum germination was observed in G.27 (20%) and in GSHV-01/1338 (28%) after 14 days incubation period.

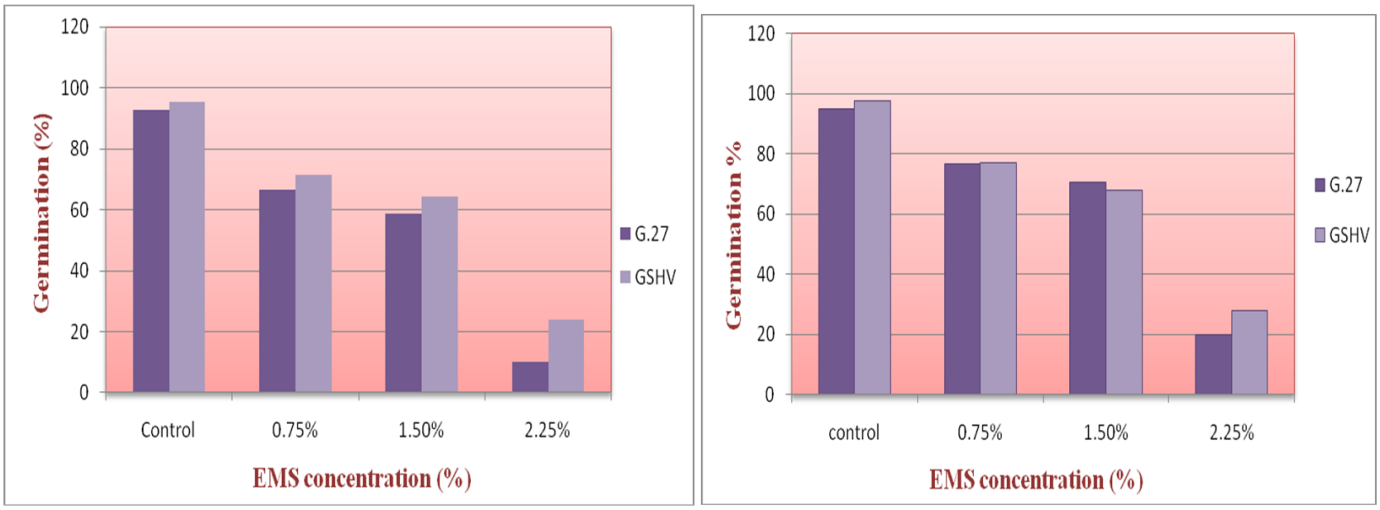

Fig. 1A. **Fig. 1B.**

Fig 1: Effect of EMS on germination percentage in cotton genotypes after incubation period of A) 7th days B) 14th days in petriplates.

Effect of EMS on root length and shoot length

From the graphs it is clear that at all the concentrations of EMS reduced the root and shoot length of cotton seeds. After 14 days and 21 days incubation period, the maximum root and shoot length was observed under control condition in both the varieties.

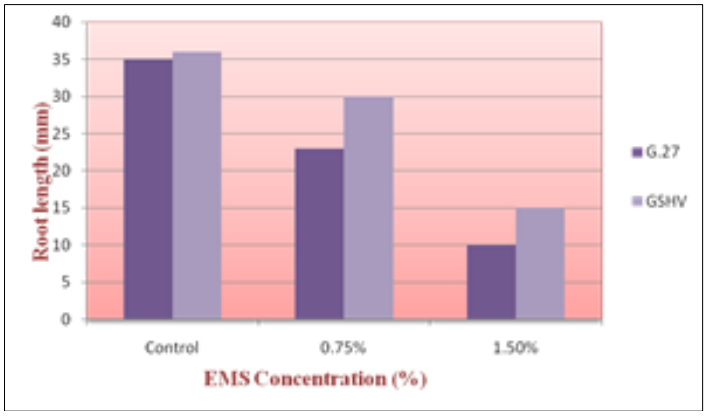

Fig. 2A

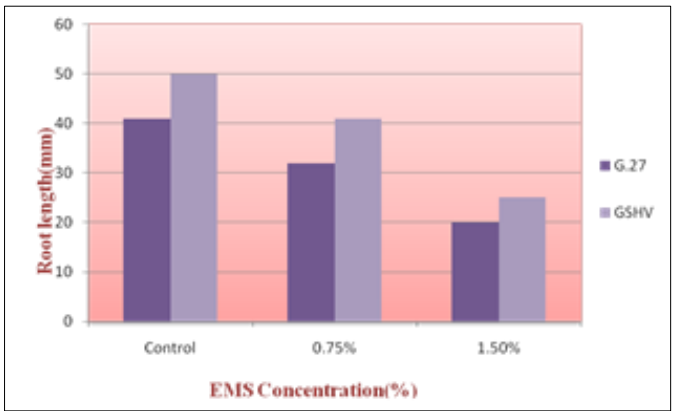

Fig. 2B

Fig 2: Effect of EMS on root length (mm) in cotton genotypes after A) 7 days of incubation period B) 14 days of incubation period

The root length was higher in GSHV-01/1338 variety (50 mm) as compared to G.27 variety (41 mm) after 21 days of incubation under controlled condition. At 0.75% concentration, maximum root length was observed (41 mm) in GSHV-01/1338 variety, where as (32 mm) was observed in G.27 variety (Fig. 2A and 2B). In a similar fashion, shoot length was also higher in GSHV-01/1338 variety (68 mm) as compared to G.27 variety (52 mm) after 21 days of incubation under controlled condition (Fig. 3A and 3B). The germination was observed in both the varieties of cotton at 2.25% concentration of EMS but could not differentiate into well developed root and shoot. This indicated that the 2.25% of concentration of EMS was lethal for cotton plant growth and development. Similar results were observed by Ali *et al.* (2012) [1] in rice for root length.

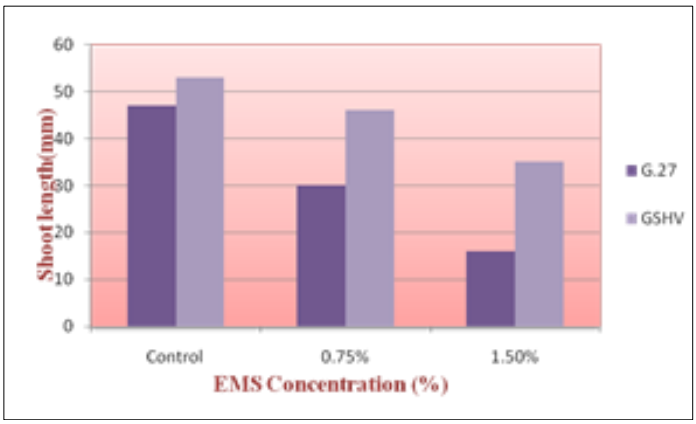

Fig. 3A

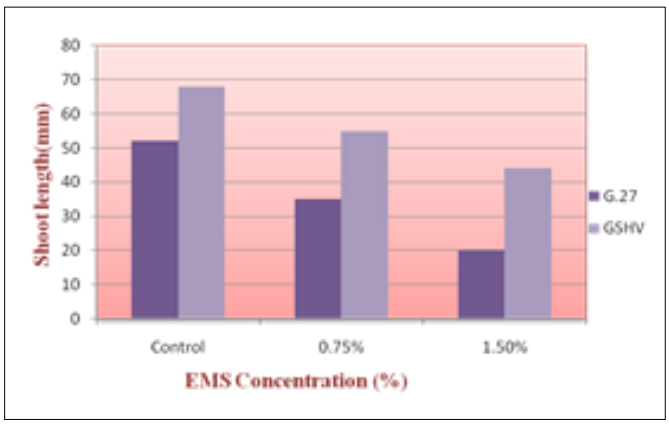

Fig. 3B

Fig 3: Effect of EMS on shoot length (mm) in cotton genotypes after A) 7 days of incubation period B) 14 days of incubation period

Estimation of genetic variability through molecular markers (RAPD, ISSR, SSR)

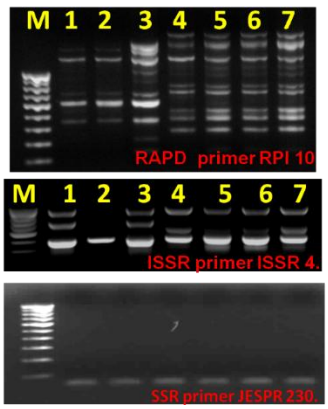

Fig 4: DNA amplification profile of RAPD primer (RPI 10), ISSR primer (ISSR 4), and SSR primer (JESPR 230). [M: 100 bp ladder; Lane (L) 1: G.27 (control); L2: G.27 (0.75%); L3:G.27 (1.5%); L4: GSHV-01/1338 (control); L5: GSHV-01/1338 (0.75%); L6: GSHV-01/1338 (1.5%); L7: GSHV-01/1338 (2.25%)].

Representative EMS treated and control of G.27 and GSHV (01/1338) were subjected for DNA profiling with 25 RAPD, 19 ISSR and 11 SSR markers. A total of 185, 184 and 186 amplicons were recorded in G.27 (control); G.27 (0.75%) and G.27 (1.5%) G.27 (1.50%) respectively. Similarly, GSHV (01/1338) (control); GSHV (01/1338) (0.75%); GSHV(01/1338) (1.5 %) and GSHV(01/1338) (2.25%) have produced 178, 179, 178 and 178, respectively (Table 1).

Table1: Number of amplicons produced by molecular markers

Varieties (Treatment)	RAPD	ISSR	SSR	Total
G.27 (control)	108	66	11	185
G.27 (0.75%)	109	64	11	184
G.27 (1.50%)	110	66	10	186
GSHV (01/1338) (control)	110	55	13	178
GSHV (01/1338) (0.75%)	110	56	13	179
GSHV (01/1338) (1.5 %)	110	55	13	178
GSHV (01/1338) (2.25%)	110	55	13	178

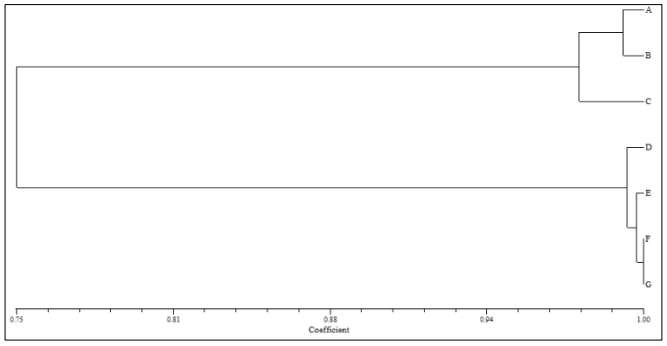

Fig 5: Dendrogram generated from UPGMA cluster analysis based on RAPD, ISSR and SSR markers [(A- G.27 (control); B - G.27 (0.75%); C - G.27 (1.50%); D- GSHV (01/1338) (control); E- GSHV (01/1338) (0.75%); F- GSHV (01/1338) (1.50%); G- GSHV (01/1338)(2.25%)]

Further, dendrogram generated by UPGMA analysis using NTSYS software revealed that G.27 (control) shares a genetic similarity of 99 per cent with G.27 (0.75%); and a similarity of 98 percent with G.27 (1.5%). Thus, resulting in 2 per cent dissimilarity among the control and treated G.27 varieties (Fig 5). Similarly, GSHV(01/1338) (control) is genetically 99 per cent similar with 0.75 %, 1.5 % and 2.25 % EMS treated representative GSHV(01/1338) varieties; resulting in 1 per cent dissimilarity (genetic distance). Thus, molecular markers used in this study are able to detect the genetic variability induced though EMS and also able to detect the genetic relationship among the varieties.

Conclusion

It can be concluded that as the increase in concentration of EMS resulted in reduction of per cent germination, root length and shoot length in both the varieties of cotton GSHV-01/1338 (*Gossypium hirsutum*) and G.27 (*Gossypium arboreum*). Further, concentration of 2.25% EMS in *G. arboreum* (G.27) found to be lethal. Further, EMS induced variation characterized through molecular markers in both the varieties of cotton reflected in variation at DNA level. However, a small portion of DNA variation has been explored through the markers used in this study. Therefore, in order to increase the precision of variation induced, these need to be further characterized with an additional set of markers preferably functional markers.

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