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Mutagenic effect of ethyl methane sulfonate (EMS) on barnyard millet (*Echinochloa frumentaceae*) variety CO(KV)2

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Abstract

Barnyard millet variety CO (KV) 2 was treated with 2 different doses of EMS (Ethyl Methane Sulfonate) (ie. 0.2%, 0.4%, 0.6%, 0.8% and 1.0%). Six days after treatment germination percentage was calculated and LD₅₀ value were fixed as 0.51%EMS. M₁ population was raised with two treatments 0.4% and 0.5%. Seedlings with normal growth and development were observed in 0.4% EMS dose and very poor establishment, stunted growth and 80% spikelet sterility was observed in 0.5% EMS treatment. In M₂ generation macro-mutants such as plant type, earliness, study stem, bigger panicle size and higher plant yield (28g/plant) were obtained.

Keywords: Barnyard millet, EMS, LD₅₀, macro-mutants

Introduction

Barnyard millet (*Echinochloa frumentacea* (Roxb.) Link) is one of the hardiest millets being cultivated for food and fodder in all types of soils and sustains adverse climatic conditions. The yield level of barnyard millet is as high as 10 t/ha in Japan, where as in India it is 1.5 to 2 t/ha. Thus, there is a greater scope for exploiting its potential under Indian conditions (Channappagoudar *et al.* (2008) [1]. In the rainfed tracts of Tamil Nadu *viz.*, Ramanathapuram, Sivagangai and Pudukottai etc. cultivation of minor millets such as Barnyard millets seems to be a viable alternative to sustain cereal food grain production under changing climatic scenario.

Availability of limited genetic variation and difficulty in hybridization makes mutation breeding as one of the viable and feasible approaches in the genetic improvement of barnyard millet. Induced mutation has been perceived as an important tool to create additional variability for quantitative and qualitative traits in a number of crop plants (Bansal *et al.* (1990) [2]. This is evident from the fact that more than 2250 varieties of different crops had been released that were derived as direct mutants or from hybridization involving desirable mutants (Ahloowalia *et al.*, (2004) [3]. In India alone, 300 and above mutant cultivars belonging to more than 55 plant species have been developed / released for cultivation.

Based on the above evidences, the present investigation was started with an aim of inducing novel variability in one of the hardiest minor millets "Barnyard millet" through mutagenesis. Chemical mutagenesis approach was followed to develop large number of mutants and mutants were screened for agronomic traits like earliness, high tiller numbers and higher yield.

Materials and Methods

A popular barnyard millet variety CO(KV)2 was used for studying the effect of mutation on germination and different agronomic traits. The chemical mutagen Ethyl Methyl Sulfonate (EMS) was used for *generating* the mutated population of CO(KV)2 barnyard millet. Initially germination test was carried out before treating of CO(KV)2 seeds with mutagen (EMS). Seeds with 10-12% moisture content were allowed for germination in petri plates with adequate amount water and around 100% germination was observed after 3 days.

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Doses of EMS were fixed as 0.2%, 0.4%, 0.6%, 0.8%, 1.0% and germination studies were evaluated with control. Hundred seed were counted for each treatment as well as untreated control and placed in separate petri-plates. Different doses of EMS was prepared and added in separate petri-plates and seeds are allowed for germination. 6 days after treatment germination percentage was calculated and LD₅₀ value were fixed. Based on LD₅₀ value treatments were fixed for generating M₁ population. M₂ seeds were sown as plant to progeny rows by adopting 30cm and 10cm spacing between rows and plants respectively. M₂ plants were monitored for different morphological mutants expressed in the population. Field experiments were conducted at Dryland Agricultural Research Station, Chettinad, Sivangangai district of Tamil Nadu.

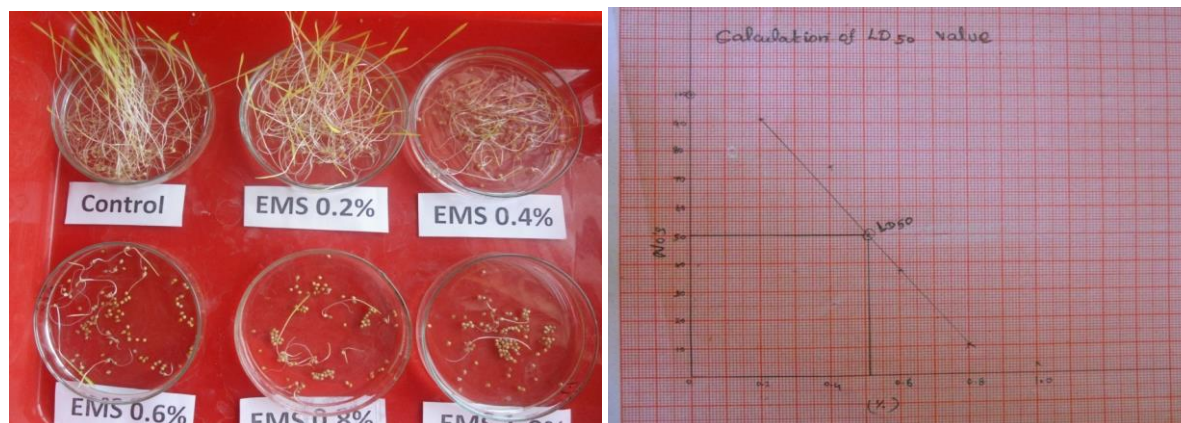


Fig 1: Effect of EMS on seed germination and LD₅₀ value in barnyard millet variety CO(KV)2 (6 days after EMS treatment)

Table 1: Germination percentage of different doses of EMS in barnyard millet

Doses	Germination percentage %
Control	100
0.2% EMS	91
0.4% EMS	74
0.6% EMS	37
0.8% EMS	10
1.0% EMS	3

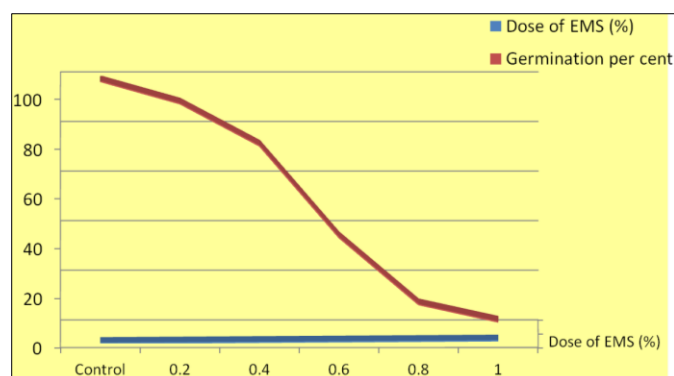


Fig 2: Different doses of EMS with germination % variation in barnyard millet variety CO(KV)2

Generation of M₁ and M₂ population

Barnyard millet variety CO (KV) 2 was treated with 2 different doses of EMS (Ethyl Methane Sulfonate) (ie. 0.4% EMS and 0.5% EMS). Since LD₅₀ value for EMS dose is fixed as 0.5%, so 0.4% EMS (slight lower dose than LD₅₀) has fixed for raising the effective mutagenic population. Initially seeds were soaked in water for 8 hours then those seeds were used for EMS treatment. Treated seeds were sown

Results and Discussion

Germination percentage and fixation of LD₅₀ value

Different doses of mutation treatments were influenced the effect on seed germination, seedlings shoot and root length. Germination status of different doses of treatments was presented in Fig 1. Around 91% germination was observed in 0.2% EMS dose. Higher doses of mutagen (0.8% EMS and 1.0% EMS) were observed with high rate of lethality (10% and 3% germination) (Table 1). Similar reports have been reported by Ambavane *et al.* (2015) [4]; Ambli and Mullainathan (2015) [5], Nivetha *et al.* (2018) [6]. Based on the germination percentage of CO(KV)2 seeds with different doses of EMS mutagen, the LD₅₀ value has been fixed as 0.51% (Fig 1 & Fig 2).

in field for raising of M₁ generation. Intercultural operations such as weeding and thinning, fertilizer application was carried out at appropriate time.

Seedlings with normal growth and development were observed in 0.4% EMS dose. Around half of the population was affected at germination stage in 0.5% EMS treatment, as well as very poor establishment, stunted growth and 80% spikelet sterility was also observed. There is no variation was observed in height and plants are harvested to raise the M₂ population. Results are concurrent with the earlier findings of Jayakumar and Selvaraj (2003) [7], Kavithamani *et al.* (2008) [8], Anju Pathania and Sood, (2011) [9]; and Ramesh *et al.* (2019) [10].

Around ten thousand M₁ seeds were sown. Approximately around 9500 individuals were obtained and CO(KV)2 was used as check. Morphological mutants were observed in M₂ population and variants were noticed such as earliness (75-80 days), purple pigmentation on stems and panicles (M₂-59-1), sturdy and large stem size, more number of tillers, compact panicle, bigger size panicle, higher seed yield/plant (28g/plant). In M₂ population, compact panicle variant M₂-149-1 (Fig.3-i) were observed and purple pigmentation on stem (Fig.3-iii), panicle. Plant with broad leaf (M₂-74-3) with 3.8cm width was observed (Fig.3-ii). Mutant M₂-56-1 identified for its sturdiness, bigger size panicle and higher single plant yield of 28 g/plant (Fig.3-iv). Variations found among M₂ individuals were due to sudden mutagenic changes by chemical ethyl methane sulfonate. Earlier studies on mutation and macro-mutants identified in M₂ generation by Raveendran (1976) [11], Yadava *et al.* (2003) [12], Nivetha *et al.* (2018) [6] and Ramesh *et al.* (2019) [10] also confirmed the results of variations found in M₂ population.

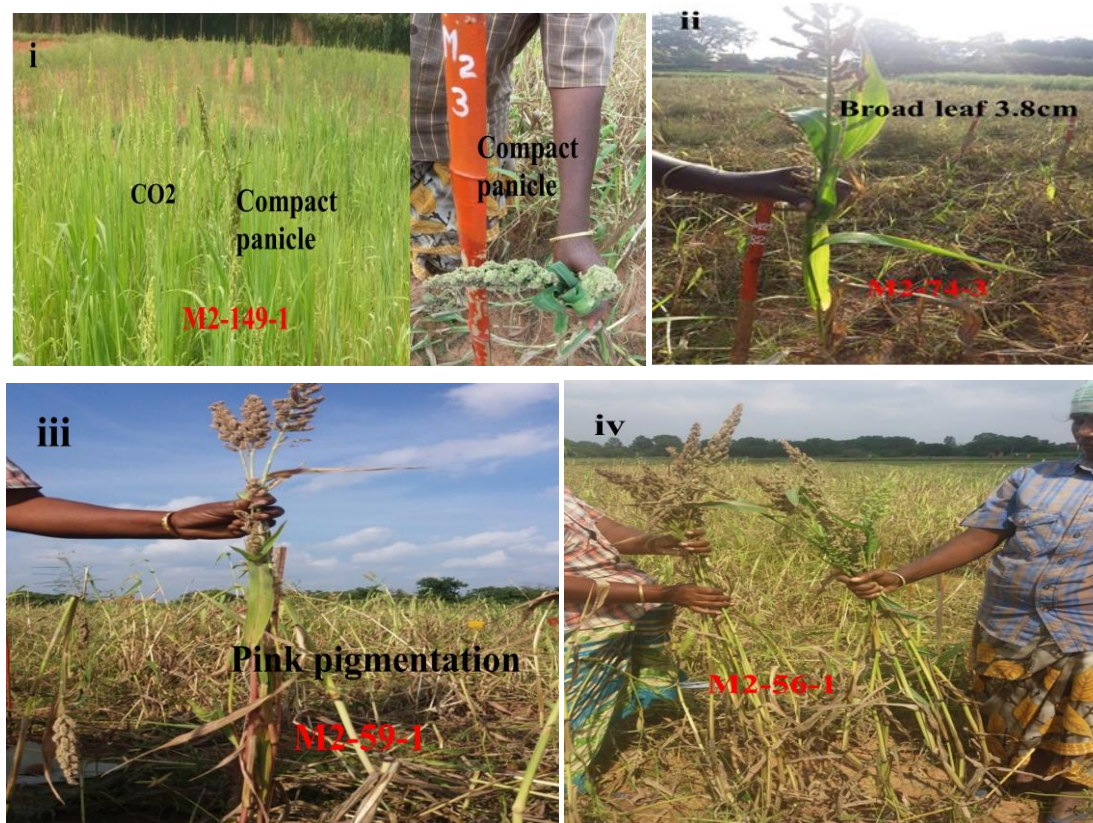


Fig 3: Variants identified in M₂ population

i. Compact panicle type, **ii.** Broad leaf mutant, **iii.** Pink pigmentation, **iv.** Sturdy stem and mutant for higher yield

Conclusion

In the present investigation chemical mutagen EMS was used to generate novel mutants in barnyard millet variety CO(KV)2. Study showed that macro-mutants with preferable phenotypic features were obtained in M₂ generation. It was additional confirmation that EMS was better chemical mutagen to create variability in barnyard millet. The mutations acquired for leaf breadth, plant type, sturdiness and bigger panicle size are further being evaluated in subsequent generation and will be utilized in barnyard millet improvement.

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