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REGULAR ARTICLE

ISOLATION OF INDUCED MORPHOLOGICAL MUTANTS IN *CAPSICUM ANNUUM* L.

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SUMMARY

Seeds of *Capsicum annuum* L. varieties Co – 1 and Ujwala were treated with two chemical mutagens viz., Ehtyl methane sulphonate and Sodium azide. Twelve different types of morphological mutants were isolated in M_2 and M_3 generations. Capsaicin content of some selected mutants was also estimated. Frequency of morphological mutants obtained was very high in variety Co -1 when compared to Ujwala.

Keywords: Ethyl methane sulphonate, Sodium azide, *Capsicum annuum* L, mutants, Capsaicin.

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1. Introduction

Mutation induction and selection methodology provides a viable additional option to plant breeders for creating useful genetic variability. It is considered as a tool that in combination with other tools such as crossbreeding can be used to achieve desired plant breeding goals. The genetic variability in a crop plant can be supplemented by induced mutations, when the germplasm sources may be inadequate to meet the plant breeding requirements. Chemical mutagens such as ethyl methanesulphonate, diethyl sulphate, sodium azide and certain base substitution and nitroso compounds appear to induce higher proportions of point mutations than chromosomal aberrations (Konzak *et al.*, 1977). Different types of morphological mutants were isolated through chemical mutagenesis in *Capsicum annuum* L. by various workers (Subhash *et al.*, 1981; Raghuvanshi and Singh, 1982; Rostaino, 1983).

In the present study, two cultivars of *Capsicum annuum* L. *viz*., Co – 1 and Ujwala were treated with different concentrations of two potent chemical mutagens, Ethyl methane sulphonate (EMS) and Sodium azide (SA). The plants were screened for morphological mutations in M_2 and M_3 generations and most of them breed true.

2. Materials and Methods

Dry seeds of *Capsicum annuum* var. Ujwala and Co – 1 were treated with Ethyl methane sulphonate (0.1%, 0.2%, and 0.3%) and Sodium azide (0.01%, 0.02% and 0.03%) solutions for 9 hrs in room temperature (27 ± 1^{0}C). The seeds were presoaked in distilled water for 12 hrs to activate embryos. The untreated (Control) seeds were kept immersed in the distilled water for the same period. Immediately after the treatment the seeds were thoroughly washed in water and sown in poly bags in order to raise the seedlings. 45 days old seedlings were transplanted to experimental field in Completely Randomized Block Designs with three replicates to raise M_1 population.

For raising M_2 and M_3 generation, 50 healthy seeds of both the varieties from each normal looking plant of all the different treatments with their respective controls were selected and sown in poly bags to raise the seedlings. Forty five days old seedlings were transplanted in the field in plant progeny rows. The spacing between plants in a row and between rows was 45 cm and 50 cm respectively in each treatment as well as in controls. The experiments were conducted in Completely Randomized Block Designs (CRBD) with three replicates.

For the estimation of capsaicin content in some selected mutants, dried chilly was powdered and extracted with ethyl alcohol. The extract was concentrated and the resulting concentrate was subjected to High Performance Liquid Chromatography (H P L C) using Shimadzu H P L C system, L C - 6A pump and SPD 6A UV detector. The C 18 chromatographic column; 25 cm x 4.6 mm id., 5 µm particle size, 40% acetonitrile and 60% DI - H_2O with 1% acetic acid (v/v) as mobile phase and a variable wavelength UV spectrophotometric detector set at 280nm. The data was compiled using Winchrom software.

3. Observations

The phenotypically visible, easily perceivable genetic alterations were observed to recover desirable mutations in M_2 and M_3 generation. The controls as well as the mutagenized populations were screened for all phenotypically detectable mutations at different stages of growth in both the varieties. Twelve different types of morphological mutants were isolated from M_2 and M_3 populations. The frequency of different mutants observed is summarized in Table 1. These mutants were categorized on the basis of the trait affected. The characteristic features of such mutants are described below: -

Tall mutants (high yielding): These mutants were isolated from 0.3% EMS treated M_3 population of the variety Co –1. These mutants were considerably taller than the control plants, their mean height was 82.7 cm where as, it was 51.49 cm for the control plants. They produced normal sized fruits and appeared at a frequency of 13.63% of the total morphological mutations.

Tall with short fruit mutants: These mutants were isolated from the 0.2% EMS treated M_3 population of the variety Co –1. These plants were characterized by the production of large number of short fruits with an average weight of 2 gm and size of 6.8 cm long and 0.9 cm width. The mean height of these mutants was 70.45 cm. Their frequency was found be 9.09%.

Tall with crinkled fruit mutants: These mutants were appeared in 0.03% SA treated M_2 population of the variety Co – 1. The mean height of this mutant was 68.73 cm whereas, it was 49.59 cm in the control plants. These mutants produced normal fruits but showed crinkled pericarp. These were low yielding when compared to the control plants. Their frequency was found to be 9.09%.

Dwarf mutants: These mutants appeared in 0.3% EMS treated M_2 population of the variety Ujwala. These mutants were conspicuous by the short stature and reduced yield components. The mean height of the control was 72.83 cm whereas, it was 48.12 cm in the dwarf mutants. Their frequency was 13.63% of the total morphological mutations.

Short fruit mutants: These mutants were observed in 0.3% EMS treated M_2 population of the variety Co –1. The average weight of the fruit was 2.75 gm. The mean fruit length and fruit width were 6.0 cm and 1.0 cm respectively. The frequency of occurrence of such mutants was 11.36% of the total morphological mutants.

Erect fruit mutants: These mutants were recovered from the 0.2% EMS treated M_3 population of the variety Co - 1. The fruits of these mutants were erectly oriented. The number of branches and yield were considerably reduced. The frequency of this mutation was 13.63% of the total morphological mutations.

Yellow slender fruit mutants: These mutants were screened from the 0.01% SA treated M_3 population of the variety Co – 1. They produced greenish yellow slender fruits with viable seeds. The average fruit weight was 1.75 gm and fruit length and width, 8.00 cm and 0.78 cm respectively. The frequency of such mutants was 4.55% of the total morphological mutants.

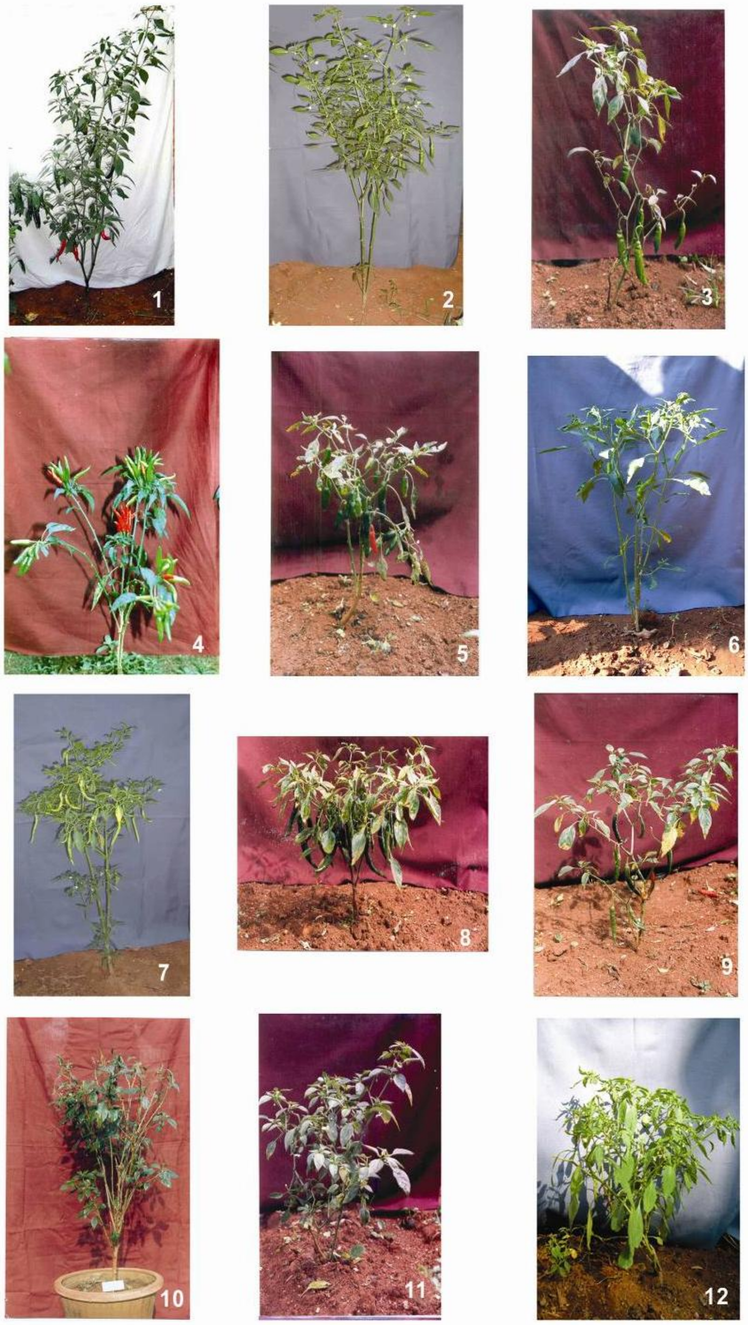
Long thick fruit mutants: The fruits of this type of mutants are comparatively longer and thicker. They appeared in the 0.02% SA treated M_2 population of the variety Co -1. The average fruit length was 11.52 cm and fruit width, 1.7 cm. The mean fruit weight was 7.00 gm whereas, 4.3 gm in control plants. The frequency of such mutants was 11.36% of the total morphological mutants.

Long slender fruit mutants: These mutants were recovered from 0.02% SA treated M_2 population of the variety Co –1. They produced longer fruits with an average fruit length of 10.98 cm and width of 1.12 cm. These mutants were low yielding when compared to the control plants. They appeared at a frequency of 4.55% of the total morphological mutants.

Seedless mutants: Two seedless mutants were isolated from the 0.03% SA treated M_2 population of the variety Ujwala. The growth of these mutants was normal but produced small sized fruits with aborted seeds. The average fruit weight was only 1.5 gm and fruit length and width, 4.34 cm and 0.84 cm respectively. The frequency of these mutations was 4.55% of the total morphological mutants.

Table 1. Frequency (%) of morphological mutants in *Capsicum annuum* after chemical mutagenesis

Sl. No.	Mutant type	Mutagen Dose	Variety	Generation	Frequency
1	Tall mutant (high yielding)	0.3% EMS	Co -1	M_3	13.63
2	Tall with short fruit mutant	0.2% EMS	Co -1	M_3	9.09
3	Tall with crinkled fruit mutant	0.03% SA	Co -1	M_2	9.09
4	Dwarf mutant	0.3% EMS	Ujwala	M_2	13.63
5	Short fruit mutant (high yielding)	0.3% EMS	Co -1	M_2	11.36
6	Erect fruit mutant	0.2% EMS	Co -1	M_3	13.63
7	Yellow slender fruit mutant	0.01% SA	Co -1	M_3	4.55
8	Long thick fruit mutant	0.02% SA	Co -1	M_2	11.36
9	Long slender fruit mutant	0.02% SA	Co -1	M_2	4.55
10	Seedless mutant	0.03% SA	Ujwala	M_2	4.55
11	Non viable mutant	0.03% SA	Co -1	M_2	2.27
12	Viable chlorophyll deficient mutant	0.2% EMS	Co -1	M_2	2.27



Figs: 1 Tall mutant , 2-Tall with short fruit mutant, 3-Tall with crinkled fruit mutant, 4- Dwarf mutant, 5-Short fruit mutant, 6- Erect fruit mutant, 7-Yellow slender fruit mutant, 8-Long thick fruit mutant, 9- Long slender fruit mutant,10- Seedless mutant, 11- Non-viable mutant, 12- Viable chlorophyll deficient mutant.

Non-viable mutant: One mutant, which failed to produce fruits was noticed in the 0.03% SA treated M_2 population of the variety Co –1. The mutant showed normal growth habit but produced less number of branches and flowers. The flower development was very late, but the anthers produced 58% viable pollen grains. The frequency of this mutant was 2.27%.

Viable chlorophyll deficient mutant: One viridis chlorophyll deficient mutant, which was isolated from the 0.2% EMS treated M_2 population of the variety Co – 1 was grown to maturity, flowered and produced viable seeds. This mutant showed slow growth rate with viridine green colour of leaves. The fruit size was comparatively smaller than the control. The mean fruit weight was only 2.31 gms and the fruit length and width, 3.48 cm and 0.99 cm respectively. The frequency of this mutant was found to be 2.27% of the total morphological mutations.

Capsaicin content

Table 2 shows the capsaicin content and pungency in SHU (Scoville Heat Unit) of variety Co -1 and its five selected mutants. No effective morphological mutants could be isolated from the variety 'Ujwala'. The tall with short fruit mutant contains maximum capsaicin (0.30%) and pungency at the rate of 46818.06 SHU. There was no much increase in capsaicin content in other mutants. The long thick fruit mutant and viable chlorophyll deficient mutant contain very low percentage of capsaicin. The long thick fruit mutant contains only 0.05% capsaicin and pungency of 7236.12 SHU. The viable chlorophyll deficient mutant has 0.01% capsaicin and pungency of 1517.00 SHU. Short fruit mutant (high yielding) and tall high yielding mutant contain 0.21% capsaicin with 33797.80 SHU and 0.20% capsaicin with 33266.20 SHU respectively.

Table 2. Capsaicin content in some selected mutants of *Capsicum annuum*

Sl. No.	Variety/Mutant	SHU Total	% of capsaicin
1	Co -1	40667.50	0.25
2	Tall with short fruit mutant	46818.06	0.30
3	Long thick fruit mutant	7236.12	0.05
4	Short fruit (high yielding) mutant	33797.80	0.21
5	Viable chlorophyll deficient mutant	1517.00	0.01
6	Tall high yielding mutant	33266.20	0.20

SHU = Scoville Heat Unit

4. Discussion

Various kinds of phenotypically visible, easily perceivable genetic alterations were observed after mutagen treatments. These morphological mutants were isolated on the basis of altered form, phenotype or morphological architecture. The frequency and spectrum of morphological mutants differed in different mutagenic treatments and also between both the varieties. The frequency and spectrum of such mutants were highest in the treated population of variety Co -1. Thakur and Sethi (1995) observed that the frequency of morphological mutants were 2-3 times more in chemical mutagenic treatments than physical mutagenic treatments in barley. Rajam and Subhash (1995) isolated different kinds of morphological mutants in M_2 generation of *Capsicum annuum* and found that mutations induced by various treatments are of significance, since variants affecting almost every feature of the plant were observed in the treated population. Though, some of the mutants were associated with undesirable traits such as reduction in yield, it could be overcome by trans-specific crosses with other cultivars or other mutants.

In the present study, the variety Co - 1 produced more frequency of morphological mutants than the variety Ujwala (Table 1). The variation of mutants produced by the two varieties indicates the role of genetic make up of plants in determining the mutational events. The difference in mutational events in two varieties could be due to the differential sensitivity of the mutant alleles responsible for various morphological characters.

Various workers isolated different types of morphological mutants of chilly. These include multilocular ovary (Rajam and Subbash, 1983) clustered bud mutant (Subhash *et al*., 1981) erect fruit mutant (Rajam and Subbash, 1984), glossy-spinach, dwarf yellow and dwarf chlorotic mutant (Alcantara *et al*., 1996). Raghuvanshi and Singh (1982) isolated five viable morphological mutants in chemical mutagen treated M_2 generation of *Capsicum annuum*. Many such morphological mutants have been extensively studied in different crop plants such as *Phaseolus vulgaris* (Marghittu, 1972), *Pisum sativum* (Blixt, 1972) and *Cajanus cajan* (Chary and Bhalla, 1988).

In the present study, out of the five mutants analyzed for capsaicin content (Table 2), Tall with short fruit mutant contains comparatively high percentage of capsaicin (0.3%). Small sized fruits contain high concentration of capsaicin while long fruits contain low concentration of capsaicin. Fruits with thin pericarp contain higher capsaicin content as compared with varieties having thick pericarp (Hosmani, 1993). In the present investigation, the long thick fruit mutant contains comparatively low percentage of capsaicin (0.05%). The high capsaicin content of tall with short fruit mutant is most probably related to fruit size and nature of pericarp. The capsaicin content is also dependent upon placental content of chilli fruits, more the placental tissue higher the capsaicin content (Ramanujan and Thirumalachar, 1966). Raghuvanshi and Kumar (1991) selected four chilli mutants with high capsaicin content after physical and chemical mutagenesis. The change in capsaicin content in chilli is also affected by change in climate (Tiwari *et al*., 2005). The degree of pungency varies, probably due to the presence of genes modifying the factor of pungency. Inheritance of capsaicin content in placental tissue is observed to be controlled by single dominant gene (Sharma and Saini, 1980). Whereas, Ramanujam and Thirumalachar (1967) were of the opinion that the inheritance of capsaicin was of polygenic nature.

According to Gottschalk (1987), an agronomically useful trait is part of a pleiotropic pattern, so it cannot be used for breeding purposes in those cases where a negative trait belongs to the same pattern. However, the morphological mutants with negative selection value in the present study can be modified through cross breeding or selection by eliminating some undesirable traits.

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