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Research Paper

EFFECT OF CHEMICAL AND PHYSICAL MUTAGENS ON MORPHOLOGICAL CHARACTERISTICS OF *BRASSICA NAPUS* L. CV. EXCEL

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Abstract

Induced mutation study was carried out to study effect of chemical and physical mutagens on different morphological characteristics in the *Brassica napus* L. cv. Excel. Physiologically similar and uniform size seeds of *Brassica napus* L. cv. Excel weretreated with different doses of chemical Ethyl methane sulphonate (EMS) and Sodium azide (SA) andphysical mutagen (gamma radiations). Dry and pre-soaked water seeds were used for the treatments of EMS and SA mutagens while dry seeds were used for gamma radiations. Mutagen treated seeds were used to raise M_1 plant population. Fully grown up M_1 population were harvested plant wise. M_2 population were raised from M_1 seeds. M_2 population were screened for different morphological variations and abnormalities. Different morphological variations / abnormalitieswere recorded with respect to shape, number of cotyledons, leaf morphological changes and abnormal and variation in siliqua in M_2 population. Different type of alternations in shape and number of cotyledons such as single cotyledon with fused cup shape, two cotyledon one with fuse cup shape and another normal, three cotyledons, bilobed leaf, abnormal bud and siliqua with three replum were recorded in EMS, SA and Gamma radiations. Sodium azide was found to induce higher frequency of morphological variations as compared to Ethylmethane sulphonate and gamma radiation.

Key words: *Brassica napus*, Sodium azide, Ethylmethyl sulphonate, Gamma radiations, morphological variations.

INTRODUCTION

Oleiferous *Brassica* (rapeseed and mustard) is an important conventional oil seed crop of the sub-continent. *Brassicas* (Rapeseed-Mustard) occupy a prominent place ranking next to the Soybean and groundnut and ranks third in global production of oilseed crops. It is also one of the most important sources of vegetable oils and protein-rich meals worldwide and fifth among economically important crops following wheat, rice, maize, and cotton (FAOSTAT, 2011). Although there is large cultivation of *Brassica* crop in different states of India, but the productivity level in India is still low. However, there is considerable scope for improving the quality and yield potential of this crop. It is the second most important edible oilseed crop in India and account for nearly 30% of the total oilseed produced in the country (Yadava *et al*., 2009). Induced mutations have been used as a powerful tool to create genetic variability for various quantitative and qualitative traits in Indian mustard *B. juncea* (Labana *et al*., 1980). Induced mutations have been accepted as useful tool in a plant-breeding programme. One of the most important roles of mutation breeding is the creation of genetic variability in quantitative traits in various crop plants (Larik, 1975, 1978; Mahla, *et al*., 1990; Shah *et al*., 1990). The variability, thus created, enhances the chances for selection of new genotypes with desired characteristics. Mutagenesis provides a unique opportunity for the improvement of oleiferous *Brassica*. Physical and chemical mutagens have been successfully used in rapeseed and mustard to evolve new varieties with improved economic traits (Kumar 1972; Kamala and Rao, 1984; Das and Rahman, 1988; Mahla *et al*., 1990, 1991; Shah et *al*., 1999; and Abbas et *al*., 2008). Combination of physical and chemical treatment used in other crops (Singh and Chaturvedi, 1987; Rakowand Raney 2003) has indicated the higher frequency of mutations in combined treatments as compared with the separate ones. In the present study, effect of two chemical and one physical mutagen were investigated on morphological characteristics in *B. napus* cv. Excel.

MATERIALS AND METHODS

Physiologically similar seeds of *Brassica napus* L. cv. Excel with uniform seeds were exposed to chemical (EMS and SA) and physical mutagen (gamma radiations). Dry and pre-soaked water seeds in DNA – synthetic phase for treatments of ethyl methanesulphonate and sodium azide and dry seeds for gamma radiations were utilised. Different concentrations/ doses of mutagens were determined on basis of LD_{50} seed germination studies. Dry Seeds were treated with EMS concentrations of 0 (control), 0.004%, 0.006% and 0.008% (v/v) for 6 hrs. Twelve and eighteen hours pre-soaked water seeds were treated with concentration of 0 (control), 0.04%, 0.06%, 0.08% EMS and 0 (control), 0.03% and 0.06% (v/v) solutions for 6 hrs. respectively. In the same way treatments of sodium azide were given and concentrations used were 0 (control), 0.005%, 0.007% and 0.009% (w/v) solutions for dry seeds, 0 (control), 0.03%, 0.06%, 0.09% (w/v), 0 (control), 0.04%, 0.06%, 0.08% (w/v) solutions for 6 hrs. Dry seeds were exposed to gamma radiations doses of 100 Gyr., 200 Gyr., 300 Gyr., 400Gyr., and 500 Gyr. After completion of treatments the seeds were thoroughly washed in running water 2-3 times to remove the excess mutagens stick to the seed coat and post-soaked for 1hr. in distilled water. After post-soaking seeds were used for analysis of different parameters Mutagen treated seeds were used to rise M_1 generation. M_1 population were harvested plant wise and M_2 population were raised from M_1 obtained seeds were screened for different morphological abnormalities.

RESULTS AND DISCUSSION:

Morphological abnormalities with respect to shape, number of cotyledons, leaf morphological changes and abnormal and variation in siliqua were scored in M_2 population. Different type of Cotyledon/ Leaf morphological changes/ abnormal bud and variation in siliqua were recorded from different concentrations/doses of EMS, SA and gamma radiations are tabulated in **Table 1** (**Plate I)**.

Cotyledon/ Leaf morphological changes:

In general seedling emerged out with two cotyledons. M_2 generation were screened for cotyledon/leaf morphological changes with reference to different type of alternations in shape and number of cotyledons such as single cotyledon with fused cup shape, two cotyledon one with fuse cup shape and another normal, three cotyledons, and bi-lobed leaf were noted after mutagenic treatments. Control exhibited normal two cotyledons. Number of mutant showing single cotyledon with fused cup shape were more than three cotyledonary mutants than two cotyledonary, one with fuse cup shape and another normal mutants. Among the all mutagens sodium azide was found to be more potent to induced cup shape single cotyledon and highest frequency (0.17) was noted in 0.03%12hrs.PSW+6hrs.SA. treatment while only one mutant with two cotyledons out of which one with fuse cup shape and another normal was noticed in 12hrs.PSW+6hrs.0.06% EMS., treatment with a frequency of 0.07%.

It was observed that, EMS failed to induce three cotyledonary mutants. Only single plant mutant was obtained fromeach concentration/dose of 18hrs.dry0.009%SA., 12hrs.PSW+6hrs.0.03%SA., 18hrs.PSW+6hrs.0.04%SA and 400 Gyr. gamma radiationshad three cotyledons.Only one plant with bi-lobed leaf was noticed in each 18hrs.dry 0.004%EMS. and 12hrs.PSW+6hrs. 0.06%SA., treatment.

Frequency of single cotyledon with fused cup shape mutant were highest (0.11%) > three cotyledonary mutant (0.07%) = bilobed leaf mutant (0.07%) > two cotyledon (one with fuse cup shape and another normal) mutant (0.06%) **{(Table 1)(Plate I)(Fig. 1)}**.

These changes might have been developed due to changes in physiological and metabolic activities during the development of primordia, which results in to the alteration of leaf morphology in plants. Leaf morphological changes induced by mutagens have also been recorded by many workers like Kothekar (1978) in *Solanum nigrum*, Sudharani (1990) in black gram, Hakande (1992), Satpute (1994) in Sunflower, Khandelwal (1996), Sonavane (2000) and Kulthe (2003) in winged bean. Sangsiri *et al*., (2005) in mungbean, Tohirah *et al*., (2009) in *Curcuma alismatifolia*, Different type of alternations in shape and number of cotyledons such as single cotyledon with fused cup shape, two cotyledon (one with fuse cup shape and another normal) were recorded for first time in induced mutational programme.

Abnormal bud variant:

Normally flowering buds of the inflorescence get developed into flowers. Abnormal bud variant was obtained in 18hrs. dry 0.004% EMS with a single plant with a frequency of 0.07%. Secondary branch of a single plant from 18hrs.dry 0.004% EMS, apical shoot get converted into single bud instead of inflorescence. This modified bud without sexual whorls get elongated and developed into an inflorescence containing 15 buds. This type of variant was recorded for the first time *brassica* induced mutation programme **{(Table 1) (Plate II - A. B. C.) (Fig. 2)}**.

Siliqua with three replum variant:

Replum is the characteristic of family Brassicaceae. Generally siliquae with single replum which results in to two compartments were present in control and all the mutagen (EMS, SA and gamma radiations) treated plants. 18hrs.dry 0.004% EMS treatment was the treatment in which single plant (0.007%) resulted siliqua with three replum which were joined at the center and resulted in to three compartments were noted **{(Table 1) (Plate II - D. E. F.) (Fig.2)}**. Siliqua with three replum variant was noted for first time in induced mutational programme.

Malode (1995) induced different morphological mutations in *Brassica carinata*. Malode (2004) induced '00' characteristics in *B. juncea* cv. pusa bold through induced mutation. Yellow seeded mutant line BJ SNM 200 showed characteristic marker associated with the presence of trichome on lower epidermis of leaf (Malode and Shelke, 2010). Besides this added biochemical character with low glucosinolate level, high oil (40.6%) percentage, represent an interesting opportunity for oil seed breeders and industrialist to produce quality oil in India in future (Shelke *et al.* 2010). Genetic diversity analysis of exotic, Indian and mutant in all twenty Brassica species using RAPD markers exhibited huge range of variations in their characteristic. Both *B. carinata* and *B. napus* show affinity towards each other as they are the closest groups in regards to morphological feature in comparison to that of *Brassica juncea*, 69-78% similarity among the two *B. napus* and one *B. carinata* genotype in cluster (Malode *et al.* 2010).

Table 1: Frequencies of different morphological mutants of *B. napus* L. cv. Excel in M_2 generation.

Type of mutants	Treatment	No. of plants screened	Frequency (%)
Single cotyledon with fused cup shape	0.03%12hrs.PSW+6hrs.SA.	1720	0.17 (3)
	0.04%18hrs.PSW+6hrs.SA.	1362	0.07 (1)
	300 Gyr. gamma radiations	1426	0.07 (1)
	400 Gyr. gamma radiations	1627	0.12 (2)
Two cotyledon one with fuse cup shape and another normal	0.06%12hrs.PSW+6hrs.EMS.	1420	0.07 (1)
Three cotyledons	0.009%18hrs. SA.dry	1530	0.07 (1)
	0.03%12hrs.PSW+6hrs.SA.	1720	0.06 (1)
	0.04%18hrs.PSW+6hrs.SA.	1362	0.07 (1)
	400 Gyr. gamma radiations	1627	0.06 (1)
Bilobed leaf	0.004%18hrs. EMS.dry	1453	0.07 (1)
	0.06%12hrs.PSW+6hrs.SA.	1420	0.07 (1)
Abnormal bud	0.004% 18hrs. dry EMS.	1453	0.07 (1)
Siliqua with three replum	0.004% 18hrs. dry EMS.	1453	0.07 (1)

***PSW** - pre-soaked in water; **hrs** – hours. (Figures in parenthesis denote number of plants.)

Fig. No. 1: Frequencies of different morphological leaf mutants of *B. napus* L. cv. Excel in M_2 generation.

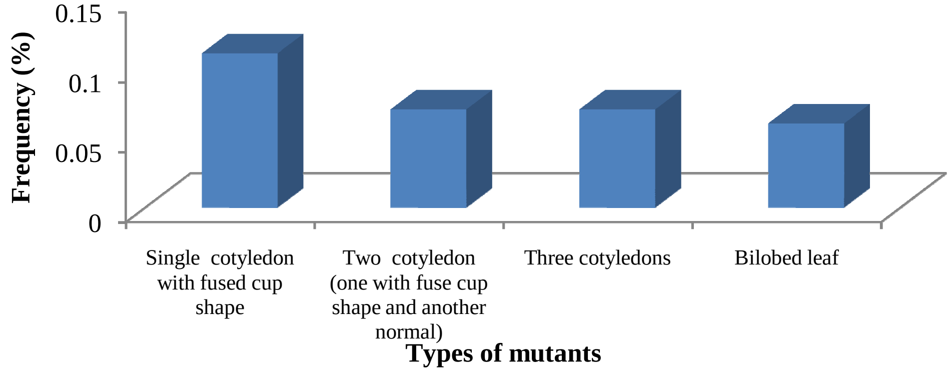
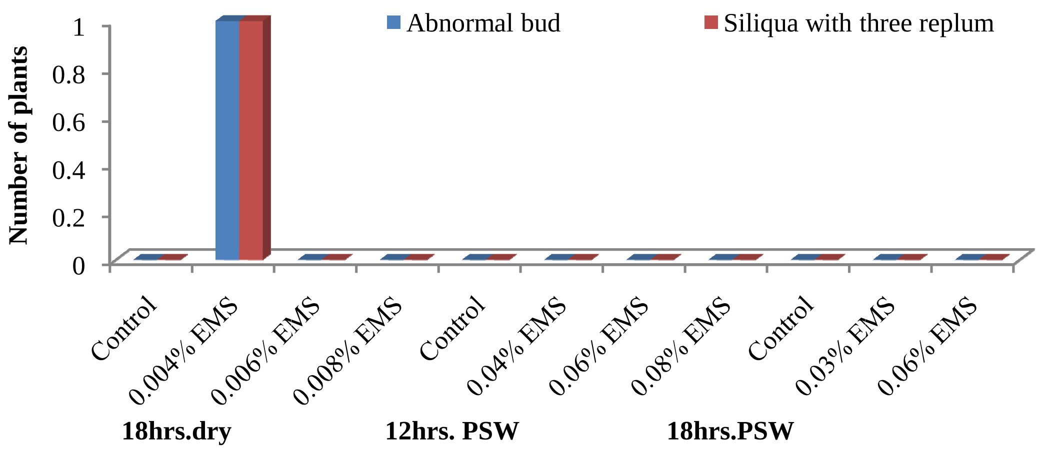


Fig. No. 2: EMS induced abnomal bud and siliqua with three replum variants in M_2 generation (*B. napus* L. cv. Excel.)



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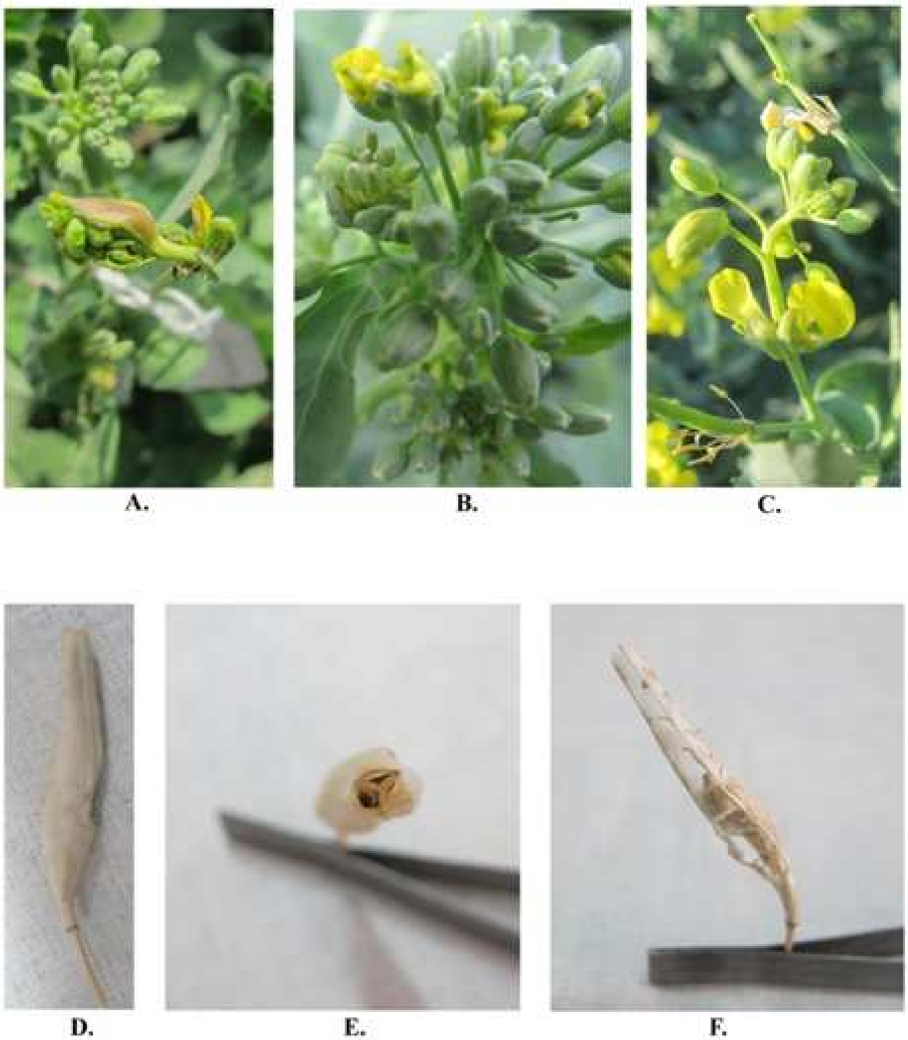
A. - Normal cotyledon. **B.** - Three cotyledons. **C.** - Bilobed leaf. **D.** - Two cotyledon one with fuse cup shape and another normal. **E.** - Photograph of two cotyledons one with fuse cup shape and another normal at two leaf stage. **F.** - Single cotyledon with fused cup shape. **G.** - Single cotyledon with fused cup shape died during further development. **H.** and **I.** - Single cotyledon with fused cup shape after few days of development.

Plate I: Photograph showing different morphological leaf mutants of *Brassica. napus* L. cv. Excel. in M_2 generation.



A. and **B.** - Abnormal bud**. C.** - Abnormal bud after few days of development.
D. E. and **F.** - Different viwes of siliqua with three replum.

Plate II: Photograph showing mutagens induced morphologicalvariants in M_2 generation of *Brassica napus* L. cv. Excel.

CONCLUSION:

In the present study, Ethylmethane sulphonate, Sodium azide and gamma radiations induced various morphological abnormalities with respect to shape, number of cotyledons, leaf morphological changes, abnormal bud and variation in siliqua in *Brassica napus* L. cv. Excel. Single cotyledon with fused cup shape, two cotyledons one with fuse cup shape and another normal, abnormal bud instead of inflorescence containing 15 buds, Siliqua with three replum were induced for the first time *Brassica* mutation breeding programme. These variant/mutants can beuse as a marker system for different biochemical or genomic characters in future *Brassica* breeding programme.

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