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Research Paper

MUTAGENIC EFFECT OF EMS ON QUANTITATIVE CHARACTERS OF *BRASSICA NAPUS* L. CV. EXCEL IN M_1 GENERATION

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Abstract

Canola (*Brassica napus* L.) is one of the important sources of vegetable oils and protein-rich meals. Canola ranks third in global production of oilseed crops and fifth among economically important crop next to wheat, rice, maize, and cotton. The present work was undertaken with the aim to investigate the effect of Ethylmethyl sulphonate (EMS) on the yield contributing characters of *brassica napus* L. cv. Excel. Physiologically similar seeds of *Brassica napus* L. cv. Excel were exposed to EMS. Dry and pre-soaked water seeds in DNA – synthetic phase for treatments were utilized. Mutagen treated seeds were used to raise M_1 generation. M_1 population was harvested plant wise. The micro mutations were recorded in M_1 generations. Quantitative characters and data for each character were recorded by taking 15 plants at random from each concentration of each treatment along with control. EMS was found to be potent to reduced plant height with increase in the yield. EMS has significant effect on number of branches per plant, number of siliquae on main axis, number of seeds per siliqua, number of siliquae per plant, single seed weight, hundred seed weight, thousand seed weight and yield per plant. EMS has no significant effect on length of siliqua. Among dry and pre-soaked water treatment of EMS, dry treatment not beneficial to increased breadth of siliqua. Among all treatment and concentrations used in present investigation, 0.006% concentration of 18hrs. dry treatment was found to be more effective to increase yield per plant.

Key words: *Brassica napus*, Mutation, Ethylmethyl sulphonate, Canola, quantitative characters.

INTRODUCTION

Canola (*Brassica napus* L.) is one of the important sources of vegetable oils and protein-rich meals. Canola ranks third in global production of oilseed crops and fifth among economically important crops next to wheat, rice, maize, and cotton (FAOSTAT, 2011). Canola oil contains 7% saturated fats which is least amount of saturated fats among the edible oils. The polyunsaturated fats in canola oil include the essential fatty acid α-linolenic acid (Omega- 3) and linoleic acid (Omega-6) which help to reduce cholesterol in the blood stream. Canola oil is also a good source of vitamins E and K and plant sterols which may to keep the heart healthy (McDonald, 2011). Therefore, for human consumption canola oil is promoted as one of the healthiest vegetable oils.

Availability of genetic diversity and genetic variation plays a critical role in developing well-adapted and improved varieties through induced mutation breeding programme. Induced mutation is an effective tool to enhance the genetic variation available to plant breeders, particularly for traits with a very low level of genetic variation (Szarejko and Forster, 2007). Several mutation breeders like Fowler and Stefansson, 1972; Ahmad *et al*., 1991; Rowland, 1991; Mac Donald *et al.,* 1991; Malode, 1995; Kott, *et al*., 1996; Bhatia *et al*., 1999; Ferrie *et al.,* 2008; *Sala et al*., 2008; Velasco *et al*., 2008: Parry *et al*., 2009 were found to develop new improved oilseed crops through successful applications of mutation breeding have been reported in the literature. Induced mutations most prominently used to generate variation that could rarely be found in germplasm collections. Mutation techniques have been also applied to improve such traits as earliness, semi dwarf, lodging resistance, disease resistance, yield and quality (Rowland, 1991; Bhatia *et al*., 1999; Parry *et al*., 2009).

According to FAO/IAEA About 3088 mutant varieties have been developed mutant varieties database (FAO/IAEA, 2011). To date, 198 different mutant cultivars of annual oilseed crops including soybean, sesame, canola, sunflower and linseed have been released (FAO/IAEA, 2011). Sathawane and Khalatkar, (1992) successfully demonstrated induction of mutation for low glucosinolate with ethyl methyl sulphonate (EMS), sodium azide (SA) and gamma radiations in *B. juncea* cv.varuna. The tasks of acclimatization and mutant production in the exotic Ethiopian mustard *B.carinata* in to Indian agro climate was effectively carried out by Malode (1995) with the objective to generate new variability, shortening of life cycle, alteration in glucosinolate, erucic acid, oil, protein and fiber content through induced mutations. The present work was undertaken with the aim to investigate the effect of Ethylmethyl sulphonate (EMS) on the yield contributing characters of *brassica napus* L. cv. Excel.

MATERIALS AND METHODS

Physiologically similar seeds of *Brassica napus* L. cv. Excel were exposed to the EMS. Dry and pre-soaked water seeds in DNA – synthetic phase for treatments were utilized. Different concentrations of EMS were determined on the basis of LD_{50} seed germination studies. For 18hrs treatments with EMS concentrations used were 0.004%, 0.006% and 0.008% (v/v) along with distilled water as a control. Twelve and eighteen hour pre-soaked water seeds + 6hrs., treatment concentrations of 0 (control), 0.04%, 0.06%, 0.08% and 0 (control), 0.03% and 0.06% (v/v) solutions respectively. In each treatment 200 seeds were treated in 200 ml. of mutagenic solution. All treatments were carried out in triplicates at 24± 0.5^0c in Remi orbital shaking incubator. 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. After washing seeds were sown in experimental field of Department of Botany, Govt. Vidarbha Institute of Science and humanities, Amravati to study various yield contributing traits. Quantitative characters and data for each character were recorded by taking 15 plants at random from each concentration from each treatment along with control. At maturity M_1 plant population were harvested plants wise. Fully mature plants were analyzed for counting of plant height (cm.), number of branches per plant, number of siliquae per plant, length of siliqua (cm), breadth of siliqua, siliquae on main axis, single seed weight, hundred seeds weight and thousand seed weight and yield per plant, was taken plant wise.

RESULTS AND DISCUSSION

During artificial induction of variability programme through induced mutations in crop plants, genetic analysis carried over with special reference to induction of drastic changes in phenotype, which is brought because of micro changes/ variability in genes. Genetic control of quantitative traits is under the control of minor genes, where each single gene contributed a little bit to the total variability. There are several reports on expression of the inheritable alterations in M_1 generation (Parkhi, 1989). These alterations have been attributed to micro and macro mutations (Gaul, 1961, 1963). In the present case, worked carried out to estimate induced variability in *B. napus* L. cv. Exel. Data on quantitative characters mean values and standard error are put forth in respective tables. Induced variability was thoroughly studied with respect to the following parameters viz. plant height (cm.), number of branches per plant, number of siliquae on main axis, length of siliqua (cm.), breadth of siliqua (mm.), number of seeds per siliqua, number of siliquae per plant, single seed weight (gm.), hundred seed weight (gm.), thousand seed weight (gm.) and yield per plant (gm.). Micro variability generated in different yield contributing traits after treatment of EMS to dry seeds for 18 hrs. (18hrs. EMS dry), 12hrs.PSW+6hrs.EMS and 18hrs.PSW+6hrs.EMS treatments with respect to control in M_1 generation are tabulated in **Tables1, 2and 3**.

Plant height: Plant height is a one of the important yield contributing character in oleiferous *Brassica*. The reduction in plant height results in an increase in grain yield. Plant height at maturity in EMS decreased as compared to control. Pre-soaked treatment of EMS showed decrease in plan height over control but increased linearly within the concentration, while such dose dependency was not observed in dry treatment though in dry treatment plant height decreased over control. In EMS treatments plant height ranges from Min. 120.71 cm. – Max. 129.75 cm. Reduced plant height (120.71 cm.) was recorded in 0.03% of 12hrs.PSW+6hrs.EMS treatment **(Table 1)**.

Kumar and Yadav, (2010) noted that plant height was found to be significantly reduced at higher doses of mutagenic treatment but some of the plants at lower doses respond positively to mutagen and recorded a slight increase in plant height. The successful utilization of sodium azide to create genetic variability in plant breeding has been also reported earlier in barley (Kleinhofs and Sander, 1975) and other crops (Avila and Murty, 1983; Micke, 1988; Routaray *et al*., 1995). Increase in plant height in case of chemical mutagens has been reported by some workers, Bose and Choudhari (1968), Bose and Gupta (1969), in *Oriza sativa* and Verma (1973) in two *Brassica* varieties with EMS. Verma (1973) stated that increase in plant height may be due to possible change in interaction of growth inhibitors and plant auxin concentration following mutation. Results of current study shows similarity with work carried out by Malode (1995) in *brassica carinata.*

Number of branches: EMS shows stimulatory effect on branch number. In all treatments of EMS, number of branches increased over control except 0.08% concentration of 12hrs.PSW+6hrs.EMS **(Tables 1, 2 and 3)**. The increase in number of branches and siliquae in mutant was recorded in *Brassica juncea* (Nayer and George, 1970). An increased in number of branches per plant was recorded in *Oryza sativa* (Bos and Choudhari, 1968 and Bose and Gupta, 1969), *Brassica carinata* (Malode, 1995), soybean (Hanafiah *et al*., 2010). Reduction in number of branches was recorded by Waghmare and Mehra (2000) in grasspea and Patil and Wakode (2011) in Soybean. Increase in number of branches after various mutagenic treatment is also recorded by Khan *et al*. (2005), Khadke (2005), Savant (2008), Bhosle and Kothekar (2008), Kashid *et al*. (2009), Satpute and Ghogare (2009).

Siliquae on main axis: Siliquae on main axis are the most productive factor and could be increased by decreasing the number of branches. It was observed that among all the concentrations of all treatments of EMS, main branch siliqua increased over control. Different treatments of EMS show significant variations in increase and decrees with respect to concentrations. Siliquae on main axis were found to be ranged in Max. 49 - Min. 63 **(Tables 1, 2 and 3)**.

Length and breadth of siliqua: Yield improvement can be achieved by manipulation of simple morphological characters. Siliqua length range in 18hrs.EMSdry treatment was 5.2 cm. to 5.5 cm. compared to control (5.1 cm.), where as in pre-soaked water treatment the range of siliqua was 4.9 cm. to 6.2 cm. and in control ranges from 5.3 cm. to 5.7 cm. **(Tables 1, 2 and 3)**. Several workers have reported enhancement in siliqua length through induced mutation. Nayer (1977) and Kumar and Das (1978) recorded stimulation in length of siliqua in *Brassica juncea* with gamma radiations. Comparative study of dry and pre-soaked water treatments of EMS shows thata pre-soaked water treatment shows more enhancements in breadth of siliqua than dry treatment over control. In case of EMS 18 hrs. dry treatment shows no effect on the breadth of siliqua as compared to control **(Table 1)**; where as in pre-soaked treatment it found to be increased over control and ranges from 6 mm. to 7 mm., while control ranges from 4mm. to 5 mm. **(Tables 2 and 3)**. Variation in siliqua size like flat siliqua, long siliqua characters have been recorded by Hakande, (1992); Sonavane, (2000); Kulthe, (2003); More, (2004); Auti and Apparao 2009; Kashid *et al.* 2009; and Selvam *et al.* 2010 in different plant systems. Broad siliqua mutants having bold seeds isolated in present work are agronomically important parameter for its improved morphological characters.

Seeds per siliqua: Number of seeds per siliqua is an important character and affects the yield of plant. Plants containing siliqua with increased number of seeds contribute to more yield. Comparative study of effect of EMS shows that number of seeds increased over control except 0.08% concentration of 12hrs.PSW+6hrs.EMS. Maximum seeds per siliqua in 18hrsdry 0.004% EMS treatment was 24 as compared to control (20). In 12hrsPre-soaked treatment seeds per siliqua were ranged from 18 - 27, compared to control (23), while in 18 hrs. Pre-soaked treatment seeds per siliqua were ranged from 22 - 27 compared to control (20) **(Tables 1, 2 and 3)**. Many workers have earlier reported variability for the seed characters through induced mutation in crop plants (Rajput, 1974) in mung bean; (Rao and Joshi, 1976) in Triticale; Gupta and Gupta (1977) in sesame; Mishra (1992) in Chickpea (*Cicer ariatinum*); Verma *et al*. (1993) in *Coriandrum sativum* L.; Singh *et al*. (1995) in linseed; Berwal *et al*.(1996) in fenugreek; Mahey *et al*. (2003) in fenugreek; Datta and Chaterjiee (2004) in fenugreek; Kumar and Gupta (2007) in black cumin (*Nigella sativa* L.).

Siliquae per plant: Number of siliqua per plant is one of the important characters for determination of high yield. Comparative effect of EMS indicates the significant variation among the treatment and concentrations. EMS treatments show that higher concentration affect negatively on siliqua per plant. Siliqua per plant ranges from Min. 145 – Max. 253, while control ranges from Min. 153 – Max. 180 (**Tables 1, 2 and 3)**. Number of siliquae per plant is related with the yield of plant. Pod length play vital role for number of seeds per pod and yield of crop. Variation in pod size like flat pod, long pod characters have been recorded by Hakande (1992), Sonavane (2000), Kulthe (2003), More, (2004); Auti and Apparao (2009) Kashid *et al.*(2009), and Selvam *et al.* (2010) in different plant systems. Broad pod mutants having bold seeds isolated in present work is agronomically important for its improved morphological characters. Causes of reduction in siliqua number were structural changes involving translocations, inversions and deletion (Caldecott *et al*., 1954; Natrajan and Ramana, 1965; Gottschalk and Baquar, 1973). Jha and Sinha (1977) obtained increased yield in *Pennisetum typhoides* following EMS treatment.

Seed weight: Seed weight is an important character for measuring the increase or decrease in yield. This character develops sequentially in time and to some extent, is interdependent (Adams, 1967). Significant variations were noted among the treatment and concentrations of EMS for the single seed weight, hundred seed weight and thousand seed weight. Highest single seed weight (0.0031 gm.) was observed in 0.08% concentration of 12hrs.PSW+6hrs.EMS treatment, compared to 0.0029 gm. of control. Maximum hundred seed weight (0.26 gm.) was observed in 0.004% concentration of 18hrs.dry EMS treatment compared to 0.26 gm. of control. Highest thousand seed weight (2.64 gm.) was observed in 0.004% concentration of 18hrs.dry EMS treatment compared to 2.58 gm. of control **(Tables 1, 2 and 3)**.

Yield per plant: EMS shows positive effect on the yield per plant which is found to be increased over control. In 18hrs dry treatment of EMS yield per plant showed significant variations in all concentration with respect to control. Maximum yield per plant (9.32 gm.) was recorded in 0.006% EMS and ranges from Min. 3.04 gm. - Max. 8.69 gm. The marked increased in yield per plant was observed over control by 5.47 gm. i.e. by 142.27% **(Table 1)**. Whereas, in 12hrs. pre-soaked treatment, show significant variations in the yield contributing characters among the concentrations all treatments shows increased in yield per plant over the control (4.11 gm.). Maximum yield per plant was recorded in 0.04% (11.10 gm.) and 0.06% (11.50 gm.) concentration **(Table 2)**. In case of 18hrs. pre-soaked treatment yield per plant decrease as increase in concentration but increase markedly over control. Maximum yield per plant (8.95 gm.) was noticed in 0.03% EMS which ranges from Min. 4.82 gm. - Max. 12.42 gm.**(Table3)**.

CONCLUSION

Present study showed that EMS was found to be potent to induce variability in yield contributing character of *Brassica napus* L. cv. Excel. It was observed that effect of EMS with respect to plant height showed reduction in plant height with increased in the yield. EMS has significant effect on number of branches per plant, number of siliquae on main axis, number of seeds per siliqua, number of siliquae per plant, single seed weight, hundred seed weight, thousand seed weight and yield per plant in M_1 generation. Among all treatment and concentrations used in present investigation, 0.006% EMS concentration of 18hrs. dry treatment was found to be more effective to increase yield per plant. In present study high heritability coupled with low genetic advance for different quantitative characters in M_1 generation of *B. napus* L. Cv. Excel indicates that the trait is controlled by non- additive gene action whereas high heritability coupled with low genetic advance for different character or trait is indicate that it is controlled by non-additive gene action. Thus variability generated in M_1 generation is useful successive generations for true breeding nature and further variability with respect to quantitative characters.

Table 1:The estimate of different variability parameters for yield contributing characters in control and concentrations of 18hrs.EMS dry treatment on *B. napus* L. cv. Excel in M_1 generation.

Characters	Parameters	Control	0.004%	0.006%	0.008%
PLANT HEIGHT (cm.)	Mean±SE	**152.34 ± 2.00**	127.30 ± 1.84	123.58 ± 2.34	127.11 ± 2.92
	Range(Mini.Max.)	104 - 152	99 - 151	92 – 145	99 - 141
NUMBEROF BRANCHES	Mean±SE	5 ± 0.67	**7** ± 0.43	6 ± 0.47	**7** ± 0.52
	Range(Mini.Max.)	03 - 8	3-15	03-12	4 - 12
SILIQUAE ON MAIN AXIS	Mean±SE	58 ± 2.65	61 ± 2.61	**62 ± 3.26**	58 ±2.45
	Range(Mini.Max.)	35 - 76	60 - 99	42 – 82	44 - 78
LENGTH OF SILIQUA (cm.)	Mean±SE	5.1 ± 0.14	5.2 ± 0.09	**5.5 ± 0.16**	5.2 ± 0.17
	Range(Mini.Max.)	03-6	3.5 - 6.3	3.6 - 6.8	3.8 - 7
BREADTH OF SILIQUA (mm.)	Mean±SE	6 ±0.03	6 ± 0.01	**6 ± 0.02**	6 ±0.03
	Range(Mini.Max.)	4-8	5 - 8	4 – 7	6-8
NO.OF SEEDS/ SILIQUA	Mean±SE	20 ± 1.08	**24 ± 0.65**	23 ±1.020	23 ± 0.88
	Range(Mini.Max.)	8 - 28	16 - 31	11 -29	17 - 31
SILIQUAE/ PLANT	Mean±SE	180 ± 25.16	**206 ± 17.10**	204 ± 24.25	196 ± 18.18
	Range(Mini.Max.)	99 - 430	80 - 498	72 – 535	102 - 389
SINGLE SEED WEIGHT (gm.)	Mean±SE	**0.0030 ± 0.00**	**0.0030 ± 0.00**	0.0025 ±0.00	0.0023 ± 0.00
	Range(Mini.Max.)	0.0019 - 0.0038	0.0024 - 0.0043	0.0016 - 0.0037	0.0018 - 0.0032
100 SEED WEIGHT (gm.)	Mean±SE	0.26 ± 0.00	**0.26 ± 0.00**	0.24 ± 0.00	0.23 ± 0.00
	Range(Mini.Max.)	0.21 - 0.29	**0.22 - 0.30**	0.20 - 0.33	0.19 - 0.26
1000 SEED WEIGHT (gm.)	Mean±SE	2.58 ± 0.05	**2.64 ± 0.06**	2.34 ± 0.07	2.28 ± 0.06
	Range(Mini.Max.)	2.21 - 2.96	**2.20 - 2.97**	1.98 - 3.08	1.86 - 2.64
YIELD PER PLANT (gm.)	Mean±SE	3.84 ± 1.18	7.63 ± 0.64	**9.32 ± 1.42**	7.74 ± 0.79
	Range(Mini.Max.)	1.70 - 9.26	3.00 -18.18	3.04 - 8.69	3.28 -16.08

± S.E. - standard error

Table 2:The estimate of different variability parameters for yield contributing characters in control and concentrations of 12hrs.PSW+6 hrs.EMS treatment on *B. napus* L. cv. Excel in M_1 generation.

Characters	Parameters	Control	0.04%	0.06%	0.08%
PLANT HEIGHT (cm.)	**Mean±SE**	**155.27±3.26**	122.50±3.03	129.75±4.13	127.80±7.41
	Range(Mini.Max.)	149-169	111-137	117-150	107-151
NUMBER OF BRANCHES	**Mean±SE**	6±0.69	**8±1.19**	**8±1.41**	5±0.4
	Range(Mini.Max.)	3-10	4-17	4-18	5-7
SILIQUAE ON MAIN AXIS	**Mean±SE**	51±3.27	58±3.08	**63±4.98**	56±7.83
	Range(Mini.Max.)	53-86	46-73	38-94	31-74
LENGTH OF SILIQUA (cm.)	**Mean±SE**	5.3±0.15	**6.2±0.22**	6.1±0.32	4.9±0.53
	Range(Mini.Max.)	3-6.6	4.5 – 7	5-6.9	4 - 6.5
BREADTH OF SILIQUA (mm.)	**Mean±SE**	5±0.02	**8±0.03**	7±0.05	6±0.09
	Range(Mini.Max.)	4 - 6	6 – 8	6 - 8	5 - 8
NO.OF SEEDS/SILIQUA	**Mean±SE**	23±1.07	**27±0.87**	**27±2.67**	18±3.67
	Range(Mini.Max.)	15-29	19-31	20-48	19-30
SILIQUAE/ PLANT	**Mean±SE**	167±28.03	225±31.54	**253±35.22**	148±36.36
	Range(Mini.Max.)	148-410	97-487	121-484	64-248
SINGLE SEED WEIGHT (gm.)	**Mean±SE**	0.0029±0.14	0.0023±0.00	0.0024±0.00	**0.0031±0.00**
	Range(Mini.Max.)	0.0022-0.0031	0.0018-0.0030	0.0014-0.0038	0.0028-0.0034
100 SEED WEIGHT (gm.)	**Mean±SE**	**0.28±0.00**	0.23±0.00	0.23±0.00	0.27±0.02
	Range(Mini.Max.)	0.24-0.33	0.20-0.29	0.20-0.29	0.20-0.31
1000 SEED WEIGHT (gm.)	**Mean±SE**	**2.73±0.06**	2.17±0.06	2.30±0.07	2.61±0.20
	Range(Mini.Max.)	2.33-3.25	1.90-2.63	1.98-2.89	1.97- 3.01
YIELD PER PLANT (gm.)	**Mean±SE**	4.11±1.13	11.10±1.74	**11.50±2.1**	10.14±2.54
	Range(Mini.Max.)	2.31-7.35	4.42-27.20	3.35-28.66	4.08-14.96

± S.E. - standard error

Table 3: The estimate of different variability parameters for yield contributing characters in control and concentrations of 18hrs.PSW+6hrs.EMS treatment on *B. napus* L. cv. Excel in M_1 generation.

Characters	Parameters	Control	0.03%	0.06%
PLANT HEIGHT (cm.)	Mean±SE	**155.61 ±4.024**	120.71 ±3.26	125.75 ±6.42
	Range(Mini.Max.)	142-169	97-140	94-150
NUMBER OF BRANCHES	Mean±SE	5 ±2.70	**8 ±0.70**	**8 ±2.68**
	Range(Mini.Max.)	04-11	3-13	4-27
SILIQUAE ON MAIN AXIS	Mean±SE	48±3.39	**58±4.57**	49±3.46
	Range(Mini.Max.)	33-68	44-72	28-62
LENGTH OF SILIQUA (cm.)	Mean±SE	**5.7±0.32**	5.1±0.22	5.0±0.23
	Range(Mini.Max.)	3.6-7.6	3.2-6	4.3-6.1
BREADTH OF SILIQUA (mm.)	Mean±SE	4 ±0.039	**7 ±0.034**	7 ±0.036
	Range(Mini.Max.)	4-6	6-8	6-8
NO.OF SEEDS/SILIQUA	Mean±SE	20±1.59	22±1.53	**27±1.43**
	Range(Mini.Max.)	9-29	12-30	20 -32
SILIQUAE/ PLANT	Mean±SE	153±82.07	**160±20.17**	145±21.45
	Range(Mini.Max.)	61-418	83-256	67-207
SINGLE SEED WEIGHT (gm.)	Mean±SE	0.0021±0.0014	**0.0027±0.0016**	0.0022±0.0017
	Range(Mini.Max.)	0.0018-0.0034	0.0018-0.0036	0.0018-0.0032
100 SEED WEIGHT (gm.)	Mean±SE	0.20±0.0069	**0.24±0.0069**	0.21±0.0084
	Range(Mini.Max.)	0.20-0.30	**0.20-0.28**	0.18-0.25
1000 SEED WEIGHT (gm.)	Mean±SE	1.94±0.069	**2.40±0.070**	2.09±0.096
	Range(Mini.Max.)	1.80-2.97	2.00-2.88	1.84-2.55
YIELD PER PLANT (gm.)	Mean±SE	4.96±2.70	**8.95±0.94**	7.40±1.29
	Range(Mini.Max.)	2.84-10.57	4.82-12.42	3.81-4.69

± S.E. - standard error

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