



INDUCED MUTAGENESIS FOR SEED QUALITY TRAITS IN ETHIOPIAN MUSTARD (*Brassica carinata* a. *braun*)

F. A. Sheikh, B. Lone, S. Najeeb, Asif B. Shikari, G. A. Parray, A. G. Rather, R. R. and R. S. Khudwani
Division of Plant Breeding and Genetics, SKUAST-K, Shalimar, Srinagar, India
E-Mail: sfarog4@yahoo.com

ABSTRACT

Present study was undertaken to broaden the genetic base in *Brassica carinata* cv. PC 5 for seed quality traits through induced mutagenesis. Mutagenesis with 0.5 per cent ethyl methane sulphonate followed by selfing in M_1 and screening for fatty acid composition through half seed method in M_2 and M_3 generations led to the identification of stable M_4 progenies having desirable fatty acid profile. One of the progeny EMS 9-56 appeared especially promising as it had about 24 percent erucic acid, 30 per cent oleic acid, and 40 percent oil content as against corresponding values of 46 percent, 11 percent and 34.2 percent in base variety, PC 5. Increase in oil content to 40.6 per cent was a significant achievement of the study as *B. carinata* germplasm lacks variability for this important trait.

Keywords: mustard, seed quality, *B. carinata*, mutagenesis, erucic acid, oleic acid, oil content.

INTRODUCTION

The Ethiopian mustard (*Brassica carinata*) is a potential oilseed crop under semi arid conditions as it possesses inbuilt resistance to drought, diseases and pests (Malik, 1990; Getinet *et al.*, 1996). Despite excellent productivity, its adoption among farmers is limited due to low oil content and poor oil and meal quality. The restricted amount of genetic variability available in natural *B. carinata* for these traits has constrained the breeding programmes aimed at improvement of the crop. Since crucifers are known to respond favorably to mutagenic treatment (Bhatia *et al.*, 1999) and fatty acid mutants have been identified in *B. napus* (Rakow, 1973; Robbelen and Nitsch, 1975) and *B. rapa* (Laakso *et al.*, 1986), the present study was undertaken to induce desired variability for quality attributes in *B. carinata* through induced mutagenesis using ethyl methane sulphonate as the mutagen.

MATERIALS AND METHODS

Brassica carinata cv. PC5 formed the base experimental material. It is a high yielding, tall, late maturing, non-canola cultivar having 34 percent oil content in its seed. About 5000 pre soaked seeds of PC 5 were treated with 0.5 per cent EMS for 12 hrs at room temperature with intermittent shaking. Treated seeds were thoroughly washed and immediately sown in the field to raise M_1 generation. M_1 plants were selfed and 10 primary pods per plant bulked to raise M_2 seed. Randomly selected 1000 M_2 seeds were evaluated for fatty acid composition following non-destructive half seed technique (Appelqvist *et al.* 1968). Seedlings derived from seeds having low erucic acid content were shifted to field to generate M_2 population. Selfed seeds of M_2 plants were again subjected to half-seed analysis to identify M_3 plants having desired fatty acid composition. Selected M_3 plants having low erucic acid and high oleic acid were selfed and harvested individually to raise seed for plant to progeny rows in M_4 generation wherein ten best progenies (for seed quality traits) alongwith check PC5 were evaluated in randomized complete block design with two replications. Standard

package of agronomic practices was followed to raise a good crop. Ten representative plants of each M_4 progeny were assessed for morphological and chemical parameters. Morphological data was subjected to analysis of variance to estimate percent genetic gain following mutagenesis. Besides fatty acid profile, harvest of M_4 progenies (M_5 seed) was evaluated for total glucosinolates content (Kumar *et al.*, 2004) and per cent oil content using nuclear magnetic resonance (Newport Analyser KM11A).

RESULTS AND DISCUSSIONS

Mean and variances with respect to fatty acid profile in the individual M_2 and M_3 seeds is presented in Table-1. As is apparent from range and coefficient of variation values for different fatty acids, mutagenesis was effective in enhancing the spectrum of variability. Mean erucic acid content decreased from 45.9 per cent in PC 5 to 36.2 per cent in M_2 , with few plants having as low as 20.5 per cent erucic acid. Selected M_2 seedlings having low erucic acid were transferred to field to raise M_2 population which was bulk harvested to produce M_3 seed. About 780 M_3 seeds were again subjected to half seed analysis for identifying seedlings with desirable fatty acid profile. Significant response to selection for low erucic acid was observed in M_3 generation as indicated by the mean estimate of 27.5 ± 0.38 percent in M_3 against the 36.2 ± 0.68 percent in M_2 (Table-1). As expected, the reduction in erucic acid was associated with an appreciation in oleic acid from 19.7 ± 0.61 in M_2 to 28.2 ± 0.25 percent in M_3 showing an increase of 8.5 percent over M_2 . The coefficient of variation in M_3 generation for oleic, linoleic, linolenic, eicosenoic and erucic acid contents were 24.7, 30.5, 53.6, 33.5 and 38.5 respectively as against 98.3, 70.3, 137.8, 56.7 and 59.4 per cent in M_2 indicating sufficient variation for selection was still present for these fatty acids (Figures 1 and 2). Similar modifications in fatty acid profile have also been observed previously in *B. carinata* following seed (Velasko *et al.*, 1995) or microspore mutagenesis (Barro *et al.*, 2001).

The fatty acid profile of M_4 progenies was evaluated from their selfed seed (M_5). Seed of ten



representative plants per progeny were analysed and the average value of these 10 estimates was taken as the fatty acid profile of the progeny (Table-2). The mean estimates for different fatty acids in M₅ generation did not show any significant change over the M₃ population. Average erucic acid content remained almost static at 27.5 and 25.4 percent over two generations. For oleic acid the estimates were 28.2 and 29.3 percent. The range of variability for oleic acid content in M₅ generation (26.4-31.6%) was lower than that observed in M₃ generation (18.9-36.8%). In comparison to M₂ and M₃ analyses, M₄ progenies recorded a very narrow range of variability inferring fixation of genes for fatty acid composition in the selected progenies. Fixation of variability for erucic acid content by M₃-M₄ generations was also observed by Velasco *et al.* (1995) in *B. carinata*. Mutagen treatment in the present study resulted in two stable mutant lines having about 25 percent (23.6 and 24.5%) erucic acid and 31.5 percent oleic acid as compared to corresponding estimates of 46 and 11 percent in the base material. Biosynthesis of erucic acid from oleic acid is mediated by two genes acting in additive fashion, each having unequal contribution (Bhat *et al.*, 2002). Mutation in any one of the two genes involved is expected to result only in a partial reduction in erucic acid level as appeared to be the case in the present situation. This also accounts for the failure of selection in M₃ to further reduce the erucic acid.

Although no direct selection for increased oil content was carried out in M₂ and M₃ generations, the mean oil content of ten M₄ (37.5 ± 0.8%) progenies showed a slight increase (3.3%) over the check PC 5 (34.2%), and four progenies possessed about 40 per cent oil content showing an increase of 6 percent. An increase of 4.5 per cent in oil percentage of *B. juncea* mutants

developed after gamma irradiation has been reported in the past (Kumar *et al.*, 2000). Mean and range for glucosinolate content (µmoles/g defatted meal) in the M₄ progenies (M₃ seed) was 93.9 ± 2.0 and 80.0-100.4 respectively, representing a marginal decline over the base material (107.6 µmoles/g defatted meal). Failure to achieve substantial reduction in meal glucosinolate content was reflective of lack of selection pressure as selection in each generation was carried out only for desired fatty acid composition. The M₄ progenies were also evaluated for their agronomic performance (Table-3). Except for one progeny (EMS 10-22), all others were at par or higher yielding than the check PC 5. High oil content (progenies EMS 6-35, EMS 7-127, EMS 8-240, EMS 9-56) or modified fatty acid composition (progenies EMS 4-70, EMS 5-12) had no yield penalty in the identified progenies. Two progenies were 8 to 11 days earlier in maturity whereas one progeny with short plant height was identified. These progenies had substantial variability as was evident from high co-efficient of variation (Table-4). Selected mutant progenies were superior to PC 5 base parent as was evident from upto 33 percent genetic advance for seed yield.

Summarizing, the present study was successful in developing diverse *B. carinata* mutant progenies having reduced erucic acid content, high oleic acid and high oil content which is a significant advance towards the development of Ethiopian mustard lines with canola characteristics. The change in fatty acid profile in the identified progenies may be ascribed to mutation in only of the two genes controlling the trait. Another cycle of mutagenesis in the germplasm developed during present investigation may help in isolating '0' erucic mutants.

Table-1. Fatty acid profile (%) of M₂ and M₃ seeds of *B. carinata* cv PC 5.

Fatty acid	Base value (PC 5)	Mean ± S.E. (Range)		Coefficient of variation (%)	
		M ₂	M ₃	M ₂	M ₃
Palmitic acid	3.5	4.2±0.23 (2.0-8.0)	3.1±0.06 (1.6- 5.9)	173.1	54.0
Stearic acid	0.6	0.3±0.05 (0.0-1.1)	0.4±0.04 (0.0 -1.3)	527.0	279.3
Oleic acid	11.2	19.7±0.61 (11.0-32.3)	28.2 ±0.25 (18.9 - 36.8)	97.9	24.7
Linoleic acid	18.1	15.2±0.36 (10.8-23.2)	18.3±0.20 (14.2 - 25.3)	70.3	30.5
Linolenic acid	11.1	12.7±0.51 (8.0-19.9)	9.9 ± 0.19 (6.6 - 14.7)	137.8	53.6
Eicosenoic acid	9.6	11.7±0.21 (5.8-14.4)	12.5 ±0.15 (9.3 - 15.2)	56.7	33.5
Erucic acid	45.9	36.2±0.68 (20.5-62.3)	27.5 ±0.38 (18.2 - 32.8)	59.4	38.5

Figures in brackets are the respective range values.

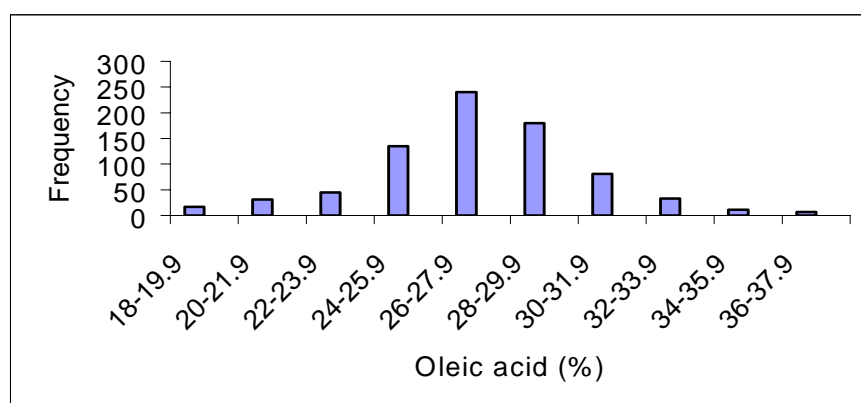


Figure-1. Frequency histogram showing variation for oleic acid content in individual seeds of M_3 generation.

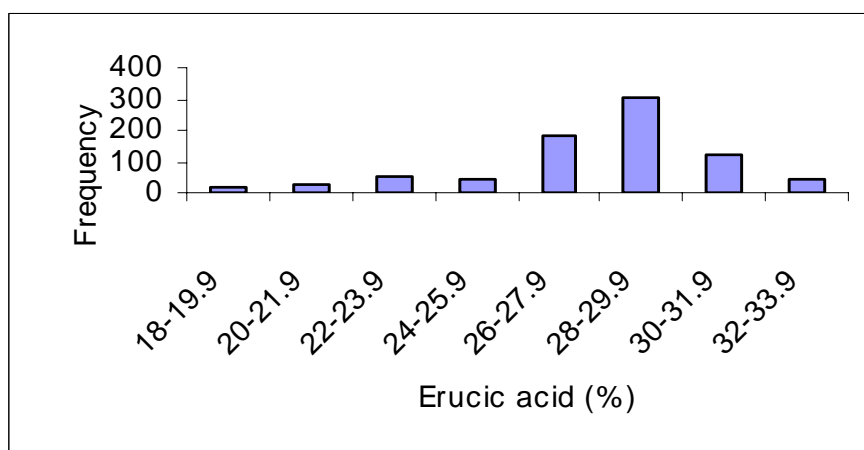


Figure-2. Frequency histogram showing variation for erucic acid content in individual seeds of M_3 generation.

Table-2. Fatty acid composition, oil and glucosinolate content in selfed seed of M_4 progenies.

Entries	Fatty acid composition (%)							Oil content (%)	Glucosinolates (μ moles/g defatted meal)
	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid		
EMS 1-23	3.6	1.0	26.8	18.9	9.3	14.2	25.8	35.4	95.8
EMS 2-85	3.7	1.0	26.4	19.5	8.8	13.2	27.2	36.4	90.7
EMS 3-76	3.3	0.8	27.9	16.0	10.4	14.0	27.5	33.5	98.3
EMS 4-70	2.9	0.6	31.0	17.2	10.0	13.0	25.1	36.0	101.2
EMS 5-12	3.8	1.4	31.6	17.8	8.8	13.4	23.2	37.6	80.0
EMS 6-35	2.8	0.4	29.4	17.4	10.8	13.2	26.0	39.1	100.4
EMS 7-127	2.9	0.7	29.6	18.0	10.2	12.5	25.9	40.3	92.8
EMS 8-240	3.0	1.4	28.7	18.8	10.3	13.8	23.9	40.6	90.3
EMS 9-56	3.7	0.7	30.2	17.0	10.9	13.0	24.5	40.5	92.3
EMS 10-22	2.9	0.9	31.5	16.7	10.0	13.3	24.5	35.6	97.5
Mean+SE	3.3+0.1 (2.8-3.8)	0.9+0.1 (0.4-1.4)	29.3+0.6 (26.4-31.6)	17.7+0.3 (16.0 - 19.5)	10.0+0.2 (8.8-10.0)	13.4+0.2 (13.0-14.2)	25.4+0.4 (23.2-27.5)	37.5+0.8 (33.5-40.6)	93.9+2.0 (90.3-101.2)
PC 5 (Check)	3.5	0.6	11.2	18.1	11.1	9.6	45.9	34.2	107.6

Figures in brackets are the respective range values.

**Table-3.** Morphological assessment of M₄ lines of *Brassica carinata* var. PC 5.

Entries	Days to flowering	Days to maturity	Plant height (cm)	Primary branches	Secondary branches	Main shoot length (cm)	Siliquae on main shoot	Siliqua length (cm)	Seeds per siliqua	Seed yield per plant (g)
EMS 1-23	119.0	169.0	166.5	26.7	63.3	40.5	12.6	4.9	12.1	11.0
EMS 2-85	109.5	170.0	189.2	26.3	95.5	39.5	17.3	5.1	14.2	15.5
EMS 3-76	108.5	163.5	170.9	23.9	56.5	34.8	16.1	4.6	11.5	10.6
EMS 4-70	116.5	166.0	159.0	27.0	100.9	35.5	15.8	4.9	12.4	14.4
EMS 5-12	93.5	164.5	196.2	25.7	66.0	35.7	17.9	4.7	12.8	12.0
EMS 6-35	108.0	165.5	171.5	21.7	55.0	35.7	19.3	4.7	13.4	11.2
EMS 7-127	108.0	164.5	184.7	24.4	70.8	40.1	13.8	4.2	11.5	11.0
EMS 8-240	105.0	160.0	202.6	26.2	82.8	39.5	19.9	4.4	13.5	13.9
EMS 9-56	97.0	157.0	204.9	23.1	82.3	36.8	18.9	4.3	11.8	13.1
EMS 10-22	108.5	165.5	176.7	24.2	35.7	41.6	15.7	4.3	11.4	7.3
PC5	105.5	167.5	185.2	21.3	59.1	36.0	17.2	5.4	15.5	11.6
GM	107.8	164.8	182.5	24.6	69.8	37.8	16.8	4.6	12.7	12.0
CD(5%)	5.6	4.8	27.0	NS	21.5	NS	3.0	0.6	NS	2.3
CV	2.3	1.3	6.6	9.7	13.8	7.5	8.0	6.3	10.8	8.5

Table-4. Phenotypic and genotypic coefficient of variance, heritability (BS), genetic advance and genetic gain for morphological traits in M₄ mutant lines.

Traits	Phenotypic coefficient of variance (%)	Genotypic coefficient of variance (%)	Heritability (%)	Genetic advance (%)
Days to flowering	6.58	6.04	84.36	11.43
Plant height	9.48	6.77	51.00	9.96
Primary Branches	10.6	4.18	15.57	3.40
Secondary Branches	29.17	25.69	77.56	46.61
Main shoot length	8.34	3.69	19.52	3.35
Siliquae on main shoot	14.66	12.26	69.93	21.11
Siliqua length	9.05	6.55	52.39	9.77
Seeds per siliqua	12.97	7.02	29.32	7.84
Days to maturity	2.45	2.07	71.55	3.61
Seed yield per plant	19.60	17.64	80.96	32.69

REFERENCES

Appleqvist L.A. 1968. Rapid methods of lipid extraction and fatty acid either preparation for seed and leaf tissue with special remarks on preventing the accumulation of lipid contaminants. *Ark. Kerici*. 28: 351-70.

Barro F., Fernandez-Escobar J., Vega M. De La and Martin A. 2001. Doubled haploid lines of *Brassica carinata* with modified erucic acid content through mutagenesis by EMS treatment of isolated microspores. *Plant Breed*. 120: 262-64.

Bhatia C.R., Nichterlein K. and Malyszynki M. 1999. Oilseed cultivars developed from induced mutations and mutations altering fatty acid composition. *Mutation Breed Rev*. 11: 1-36.

Bhat M.A., Gupta M.L., Banga S.K., Raheja R.K. and Banga S.S. 2002. Erucic acid heredity in *Brassica napus* (L.) cross-some additional information. *Pl. Breed*. 121: 456-458.

Getinet A., Rakow G. and Downey R.K. 1996. Agronomic performance and seed quality of Ethiopian mustard in Saskatchewan. *Can. J. Pl. Sci*. 76: 387-92.



Kumar A., Haider Z.A. and Varade P.B. 2000. Influence of gamma irradiation on oil quality and quantity in *Brassica juncea*. J Res. 12: 7-10.

Kumar S., Yadav S.K., Chauhan J.S., Singh A.K., Khan N.A. and Kumar P.R. 2004. Total glucosinolate estimation by complex formation between glucosinolates and tetrachloropalladate (II) using ELISA Reader. J. Food Sci. Technol. 41: 63-65.

Laakso I., Hovinen S. and Hilteunen R. 1986. Selection of high linoleic acid content in summer turnip rape (*Brassica campestris* ssp. *oleifera* var. *annua*). Acta Agriculturae scandinavica. 36: 347-351.

Malik R.S. 1990. Prospects for *Brassica carinata* as an oilseed crop in India. Exp Agric. 26: 125-29.

Rakow G. 1973. Selection of linoleic and linolenic fatty acid in rapeseed with mutation breeding (In German) Z pflanzenzuecht. 69: 62-68.

Rucker B. and Robbelen G. 1997. Mutants of *Brassica napus* with altered seed lipid fatty acid composition. Proc. 12th Int. Sym. Plant Lipids, Toronto, Canada. pp. 316-318.

Robbelen G. and Nitsch A. 1975. Genetical and physiological investigations on mutants for polyenoic fatty acid in rapeseed *B. napus*. Z. pflanzenzucht. 75: 93-105.

Velasco L., Fernandez-Martinez J. and De Haro A. 1995. Isolation of induced mutants in Ethiopian mustard (*Brassica carinata*) with low levels of erucic acid. Plant Breed. 114: 454-56.