Madras Agric. J., 99 (4-6): 228-231, June 2012
https://doi.org/10.29321/MAJ.10.100052

Genetic Enhancement of Variability Through Induced Mutagenesis in Two Genotypes of *Brassica napus* L.

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The present study deals with the extent of variability for yield, yield components and oil content in the 24 gamma-ray induced mutants (M$_7$ generation) of two genotypes of *Brassica napus* L. A highly desirable shift in mean values in the mutants as compared to national check, GSL-1 was observed for almost all the characters. The highest range of variation was recorded in grain yield (3.58-9.46 g) followed by number of primary branches (5.2-12.3), 1000-grain weight (1.76-3.93 g) and no. of siliquae per plant (113.10- 236.1). The maximum value of PCV was obtained for number of grains per siliqua followed by grain yield per plant and 1000-grain weight. The characters, such as, days to 50 per cent flowering, days to maturity, length of siliqua, plant height, number of primary branches, grain yield per plant and 1000- grain weight showed high heritability (more than 80%). Among component traits, plant height, length of siliqua, no. of grains per siliqua, no. of siliquae per plant and 1000 – grain weight showed strong positive correlation with seed yield per plant. Mutants, such as, GSM-1, GSM-2 and GSM-14 showed enhanced level of oil content, test weight and grain yield. The isolation of certain promising mutants signified the role of mutation breeding in enhancing the genetic variability in *Brassica napus L*

Key words: Mutants, *B. napus* L., *gobhi sarson*, heritability, oil content.

Among the oilseed crops, rapeseed-mustard is the third leading source of vegetable oil in the world after soybean and oil palm (USDA 2011). Globally, India accounts for 21.7 and 10.7 per cent of the total acreage and production, respectively. *Brassica napus* L. (*Gobhi sarson*), a member of the genus *Brassica*, is an allotetraploid having two genomes, namely, 'A' genome from *B. rapa* ($2n = 2x = 20$) and 'C' genome from *B. oleracea* ($2n = 2x = 18$). The seed contains up to 48 per cent of edible canola oil. After oil extraction, the resultant meal is high protein animal feed. In India, *B. napus* is grown in limited areas of Punjab, Haryana, Himachal Pradesh, etc. and suffers from long maturity duration (140-150 days) as compared to 125-135 days in mustard, tallness leading to lodging, poor test weight (2-3gm per 1000-grain), grain shattering, susceptibility to Alternaria leaf blight, powdery mildew, aphids and abiotic stresses, such as, drought and salinity, lack of genotypes with wide adaptability as well as suitable for late planting conditions under eastern parts of India.

Existing varieties as well as germplasm pool of *B. napus* have at present very limited genetic variability with respect to yield and yield components. So, it is highly imperative to induce and enhance genetic variability in the existing cultivars through mutagenesis. Hence an experiment on induction of mutants in two *B. napus* lines (GSL-1 and HNS-9601) was initiated at this laboratory during 2004-05 using gamma rays; the study led to the isolation of large number of mutants of economic significance.

Materials and Methods

The experimental materials for the present study consisted of 24 gamma-ray induced mutants of two genotypes of *B. napus*, namely, GSL-1(national check) and HNS-9601. The promising mutants were maintained at Institute of Agricultural Sciences, Banaras Hindu University, Varanasi by Dr. H. Kumar. The present experiment was carried out during *Rabi* 2009-10 at the Experimental Research Farm of the Institute. The mutants along with check, GSL-1 were evaluated in Randomized Block Design (RBD) with three replications. Each genotype was grown in single row of 3 m length, row to row and plant to plant distance was maintained at 45 and 10 cm, respectively. All the recommended agronomic practices were adopted to raise a good crop.

Ten plants were randomly selected and tagged before flowering from each genotype in each replication for data recording on ten quantitative characters, namely, days to 50 per cent flowering, plant height, number of primary branches per plant, length of siliqua, number of siliquae per plant, number of grains per siliqua, grain yield per plant, 1000- grain weight and oil content. The oil content in grains was estimated following the Soxhlet's

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*Corresponding author email: hriday_bhu@rediffmail.com method. A few seeds from sample plants were bulked and two composite samples (each of 2-3g) were drawn from each of the three replications.

The oil content was estimated using the following relationship

Oil content (%) = ($W_2 – W_3$) x 100/ ($W_2 – W_1$)

Where, W_1 = Weight of filter paper (container)

W_2= weight of filter paper + weight of initial dried seeds (crushed)

W_3= Weight of filter paper + weight of dried seeds (crushed) after oil extraction.

The statistical data of sampled plants were averaged to get mean values. The character means for each replication were then utilized for various statistical analyses; usual statistical procedures were adopted to calculate mean, range, co-efficient of variation and critical difference. The mean value of characters was subjected to analysis of variance (ANOVA) following Panse and Sukhatme (1967). The correlation coefficient was estimated following Hayes *et al.*(1955). The heritability and genetic advance were estimated following Johnson *et al*. (1955).

Results and Discussion

Gobhi sarson (*Brassica napus* L.) is a comparatively recent crop introduction as compared to other *Brassicas* and it has shown excellent performance in regions with cooler climatic conditions. However, to facilitate the spread of *Gobhi sarson* in the country, the breeding focus is now shifted to development of varieties keeping in view the location specific problems associated with *B. napus*. At present, there is very limited genetic variability available in this crop; only seven promising varieties (5) /hybrids (2) have been released in India and that too suffer from narrow adaptability apart from other inherent drawbacks. The scope of improving *B.napus* through intervarietal hybridization is limited owing to lack of genetic variability. Mutation breeding appears to be the one of the alternatives to improve this crop through enhancing genetic variability of traits of economic importance. The treatment variance of each character (except for number of grains per siliquae) was found to be significant indicating the presence of adequate variability among the mutants.

Range and Mean

The mutants showed a wide range of variability in both the directions as evident from the range and coefficient of variation (CV) coupled with shift in mean value as compared to the check, GSL-1 (Table 1). The highest range of variation was recorded in grain yield (3.58-9.46 gm) followed by number of primary branches (5.2-12.3), 1000-grain (1.76-3.93 gm), number of siliquae per plant (113.10- 236.1), days to 50 per cent flowering (64- 104) and number of seeds per siliqua (12.26 -21) with the mean of 6.52, 7.63, 2.75, 186.88, 90.10 and 20.17, respectively.

The negative shift in mean for days to 50 per cent flowering, days to maturity and plant height was considered desirable. Most of the mutants were early flowering. A total of 8 mutants matured in 140 days as compared to the check, GSL-1 (146 days). Four mutants showed significantly reduced plant height as compared to the check GSL-1; the mutant GSM-23 was the shortest in plant height (136 cm) followed by the mutants GSM-18 (160 cm) and GSM-20 (166 cm). The number of primary branches was significantly high in the mutants GSM-1 (12.30) and GSM -24 (10.53) as compared to check, GSL-1 (8.83). The lengthier siliqua was found in the mutants GSM1 (6.4cm), GSM-6 (6.70 cm), GSM-8 (6.86 cm) and GSM-19 (6.53 cm) as compared to GSL-1 (5.77 cm). The mutant GSM-22 showed the highest number of grains per siliqua (21) as compared to the check GSL-1 (19.83). The mutants GSM-23 (236.1), GSM-12 (251.8) and GSM-17 (229.9) showed significantly higher number of siliquae per plant as compared to the check GSL-1 (157). A total of seven mutants, namely, GSM -1, GSM -2, GSM-14, GSM-15, GSM -18, GSM -21 and GSM - 22 showed more than 40 per cent oil as compared to check, GSL-1 (37.7). Grain weight was significantly high (more than 3.0 gm per-1000 seed) in mutants GSM -1, 2, 6, 8, 14 and 21. Certain mutants, such as, GSM-19 (9.46), GSM-3 (8.84), GSM-4 (8.66), GSM-6 (8.4) showed significantly increased grain yield per plant as compared to the check, GSL-1 (5.97g).

Mutagenesis has been found to cause both morphological abnormalities (Grover and Tejpaul, 1979) and genetic changes in yield and yield contributing traits (Muhammad *et al*, 2007 and Kumar *et al*., 2011). A marked reduction in mean value coupled with positive shift in means of primary branched per plant, number of siliquae per plant, no. of seeds per siliqua, 1000-grain weight and grain yield per plant in Indian mustard was reported by Khan *et al*. (2008). High amount of variability were also reported by various workers, such as, Khatri *et al.* (2005) and Muhammad *et al*. (2007) in *B. juncea*, Khan *et al*. (2008) in *B. napus.*

Genotypic and Phenotypic Co-efficients of Variation

The values of genotypic and phenotypic coefficients of variation along with heritability and genetic advance are presented in Table 2. In general, as expected, phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV). The magnitude of both GCV and PCV varied with the traits. Maximum value of PCV was obtained for number of seeds per siliqua followed by grain yield per plant and grain weight. Days to 50 per cent flowering, days to maturity, length of siliqua, plant height, number of primary branches, grain yield per plant and 1000- seed weight showed high heritability (more than 80%). Plant height and number of

Table 1. Performance of twenty-four *Brassica napus* mutants for ten quantitative traits.

Mutants	Traits									
	DF	DM	PH	NPB	LS	SPS	NSP	TW	OC	SYP
GSM-1	91.33**	140.33**	187.3	6.43	6.44**	16.53	171.66	3.30**	40.69**	6.87*
GSM-2	90.33**	140.66**	193.2	6.40	6.16	16.10	164.40	3.93**	41.83**	6.95*
GSM-3	101.66	145.00	170.7	7.66	5.83	17.66	140.13	2.36	39.03	4.70
GSM-4	66.66**	143.00**	198.7	8.16	6.16	19.86	183.33**	2.83**	39.99*	8.84**
GSM-5	91.00**	142.33**	205.3	7.96	5.93	19.60	212.83**	2.36	39.62	8.66**
GSM-6	85.00**	140.33**	199.3	6.80	6.70**	20.43	196.80**	3.76**	37.70	8.40**
GSM-7	64.33**	143.00**	181.9	7.36	5.60	15.53	150.60	2.70	39.25	3.58
GSM-8	91.33**	140.66**	193.8	8.03	6.86**	20.93	209.63**	3.40**	39.03	6.29
GSM-9	88.66**	140.66**	186.7	5.63	5.63	14.30	209.36**	2.86**	39.70	4.60
GSM-10	101.33	141.66**	182.8	7.56	4.76	16.06	163.00	2.93**	38.87	5.79
GSM-11	100.66	142.33**	181.1	8.16	6.13	17.23	178.00**	2.53	37.86	8.55**
GSM-12	91.66**	140.66**	182.8	9.26	5.46	18.23	251.86**	2.16	39.79	7.72**
GSM-13	104.66	143.66**	181.6	12.3**	5.13	13.91	113.10	2.36	38.73	4.02
GSM-14	93.00**	140.66**	200.9	7.50	5.93	18.33	174.60	3.00**	40.87**	7.73**
GSM-15	90.00**	141.00**	186.3	6.90	5.53	16.80	218.30	2.57	40.21**	5.36
GSM-16	85.00**	141.66**	175.1	5.26	6.16	18.96	172.96	2.46	38.76	6.59
GSM-17	63.66**	142.00**	187.6	8.10	4.70	12.26	229.90**	2.66	38.43	5.04
GSM-18	101.66	144.66*	160.0*	7.30	5.23	13.00	113.13	1.76	40.19**	3.50
GSM-19	101.33	144.33**	193.7	8.56	6.53**	19.47	195.33	2.23	39.63	9.46**
GSM-20	97.33**	142.00**	166.0**	7.36	5.60	15.83	179.50	2.10	39.37	6.46
GSM-21	83.00**	143.33**	192.9	6.50	5.40	13.20	168.73	3.16**	40.00*	7.64**
GSM-22	88.66**	141.00**	181.9	5.76	5.80	21.00	153.56	2.93**	40.75*	5.36
GSM-23	91.33**	140.33**	136.4**	7.73	6.10	18.16	236.10**	3.33**	39.89	7.16**
GSM-24	102.00	144.33*	171.1*	10.5**	5.66	15.96	218.70**	2.50	38.43	5.51
GSL-1	100.33	146.33	180.0	8.83	5.77	19.83	157.10	2.36	37.72	5.97
Mean	90.10	142.34	184.1	7.63	5.84	20.17	186.88	2.75	39.41	6.52
Range	64-104	140-146	136-205	5.2-12.3	4.7-6.7	12.26-21	113.10-236.1	1.76-3.93	37.72-41.83	3.58-9.46
CV	1.58	0.44	3.53	8.63	3.69	30.72	5.03	5.94	3.90	5.74
CD (5%)	2.84	1.26	7.06	1.32	0.45	NS	18.81	0.36	2.23	0.77
CD (1%)	3.79	1.68	9.38	1.76	0.59	NS	25.02	0.48	2.97	1.02

DF = Days to 50% flowering, DM = Days to maturity, PH = Plant height (cm), NPB = No. of Primary branches,
LS = Length of siliqua (cm), SPS = No. of grains per siliqua, NSP = No. of siliquae per plant, OC = Oil Content (%),
TW = 1000- grain weight (g), SYP = grain yield (g)/plant; *, ** significant at 1 and 5% level of significance, respectively.

siliquae per plant showed moderate heritability, while a low level of heritability was showed by number of seeds per siliqua and oil content.

Table 2. Estimates of genetic parameters for twenty-four *B.napus* mutant lines.

Traits	GCV	PCV	h^2 (%)	G.A. as % of Mean
DF[@]	12.81	12.86	97.76	26.09
DM	1.20	1.24	83.19	2.25
PH	2.19	3.22	46.66	3.09
NPB	18.79	19.75	76.01	33.78
LS	9.17	9.563	80.56	17.05
NSP	33.51	76.42	07.35	5.91
SPS	19.07	22.15	48.91	8.6192
OC	1.68	3.86	19.05	15.13
TW	18.28	18.84	23.85	18.65
SYP	25.99	26.325	98.62	53.15

[@]DF = Days to 50% flowering, DM = Days to maturity, PH = Plant height (cm), NPB = No. of Primary branches, LS = Length of siliqua(cm), SPS = No. of grains per siliqua, NSP = No. of siliquae per plant,
OC = Oil Content(%), TW = 1000-grain weight (g), SYP = grain yield (g)/plant

High genetic advance as percent of mean was obtained for seed yield and number of primary branches. Whereas moderate values were obtained for days to 50 per cent flowering, length of siliqua, oil content and grain weight. Very low values of genetic advance were observed for days to maturity and plant height. The GCV provides a measure for comparison of the genetic variability and sometimes give an idea regarding validity of the traits under selection. However, it does not provide a clear picture of the extent of genetic gain to be expected from selection based on phenotypic basis of traits unless heritable fraction of variation is not known (Burton, 1952). A character exhibiting high broad sense heritability might not necessarily give high genetic advance .Gandhi *et al.* (1964) reported that high heritability accompanied by high genetic advance was found to be more reliable in deriving any valid conclusion. Therefore, selection should not be based solely on heritability (broad sense) but due consideration should be given to genetic advance as well.

Increase in heritability values in mutagenic population were also reported by Labana *et al.* (1980) in *B. juncea*. They found high estimates of heritability and genetic advance for plant height, number of grain per siliqua, grain yield per plant. Mahla *et al.* (2003) reported very high heritability for seed yield per plant, test weight, number of siliquae per plant. Rai *et al.* (2005) found high heritability for most of the traits except days to maturity, days to 50 per cent flowering and oil content. Khan *et al*. (2008) reported high heritability and genetic advance as percent of mean for number of siliquae per plant, test weight and grain yield per plant in *B. napus*.

Association between yield and yield contributing traits

In the 24 mutants of *B. napus*, plant height, length of siliqua, number of seeds per siliqua, number of siliquae per plant and test weight showed significantly positive correlation with grain yield per plant (Table 3). Among component traits, plant height showed significant and positive correlation with length of siliqua and test weight. Days to maturity showed a highly significant positive correlation with number of primary branches but a highly significant negative correlation with oil content and test weight.

Number of primary branches showed significant and positive correlation with days to 50 per cent flowering, days to maturity. Length of siliqua showed significant positive correlation with number of grains per siliqua, no. of siliquae per plant and grain weight, while it was negatively correlated with number of primary branches. Oil content did not show significant positive correlation with any of the yield contributing traits

Table 3. Linear correlation coefficient among ten quantitative characters in mutants of *B. napus* L.

Traits	DM	PH	NPB	LS	SPS	NSP	TW	OC	SYP
DF	0.777**	-0.3608**	0.3570**	-0.0388	0.1919	-0.3325**	**-0.3144	-0.0354	0.0755
DM		-0.0491	0.3697**	-0.1258	0.2059	-0.2104	-0.5325**	-0.4219**	-0.1304
PH			-0.1261	0.2897*	0.1691	0.2023	0.3005**	0.0244	0.4372**
NPB				-0.2725*	0.0984	-0.1027	-0.3853**	-0.3337**	-0.1017
LS					0.3620**	0.2982**	0.4508**	-0.0007	0.5992**
SPS						0.0879	-0.1611	0.0239	0.429**
NSP							0.2071	-0.168	0.4844**
TW								0.2182	0.2727**
OC									-0.0089

DF = Days to 50% flowering, DM = Days to maturity, PH = Plant height(cm), NPB = No. of Primary branches, LS = Length of siliqua(cm), SPS = No. of grains per siliqua, NSP = No. of siliquae per plant, OC = Oil Content(%), TW = 1000- Grain weight (g), SYP = Grain yield (g)/plant *, ** significant at 1 and 5% level of significance, respectively.

Length of siliqua, number of siliquae per plant, number of grains per siliqua and plant height were found to exhibit significant positive correlation with seed yield. Earlier workers have reported similar relationship (Mitra *et al*., 2006 and Marjanovic *et al.*, 2008). Number of primary branches per plant and siliquae per plant exhibited significant positive correlation with yield per plant (Rai *et al.*, 2005 and Mitra *et al.*, 2006). Earlier workers reported both positive (Mitra *et al.*, 2006) as well as negative correlation (Muhammad *et al.*, 2007) of grains per siliqua with seed yield per plant. Test weight showed highly significant and positive correlation with grain yield per plant. Both positive (Mitra *et al.*, 2006) and negative correlation (Kumar and Srivastava, 2004) between grain weight and seed yield per plant were reported.

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Received: December 26, 2011; Accepted: April 10, 2012