Pak. J. Bot., 41(3): 1189-1195, 2009.

INDUCED QUANTITATIVE VARIABILITY BY GAMMA RAYS AND ETHYLMETHANE SULPHONATE ALONE AND IN COMBINATION IN RAPESEED (*BRASSICA NAPUS* L.).

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Abstract

Genetic variability induced by gamma rays (750 Gy, 1000 Gy) and ethylmethane sulphonate (EMS) (0.75%, 1.00% solution) alone and in combination (750 Gy+0.75% EMS, 750 Gy+1.00% EMS, 1000 Gy+ 0.75% EMS and 1000 Gy+1.00% EMS) was evaluated for important economic character in rapeseed (*Brassica napus* L.) cv. Waster. Comparison with control revealed that the mutagen treatments shifted the mean values in positive as well as negative directions. However, all the mutagens showed enhancing effect for siliquas per plant and deteriorating effect for grains per siliqua and oil (%). Combinations of physical and chemical mutagen have shown enhancing effect on primary branches. There was a considerable increase in variance for all the traits under study. The induced variation can be exploited in the evolution of new varieties of rapeseed with improved agronomic traits.

Introduction

Induced mutations have been accepted as useful tool in a plant-breeding programme. One of the most important role of mutation breeding is the creation of genetic variability in quantitative traits in various crop plants (Dixit & Dubey, 1985; Khan 1983; Ahmed & Ahmed 1979; Larik, 1975b, 1978; Larik *et al*., 1980 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 (Mahla *et al.*, 1990, 1991; Rehman, 1990; Rehman *et al*., 1987; Das & Rehman, 1988; Kumar 1972; Kamala & Rao, 1984; Shah *et al*., 1999; Genet *et al.*, 2005 and Abbas *et al.*, 2008). Combination of physical and chemical treatment used in other crops (Singh & Chaturvedi, 1987; Chang & Gao, 1988; Rakow & Raney 2003) has indicated the higher frequency of mutations in combined treatments as compared with the separate ones.

The present studies were conducted to compare the relative effectiveness of gamma rays and ethylmethane sulphonate (alone and in combination) to induce genetic variability in the rapeseed (cv. Waster) and to isolate the genotypes with improved characters. This paper discusses the magnitude of induced changes with particular reference to genetic parameters viz., phenotype and genotype coefficient of variation, heritability and genetic advance.

Materials and Methods

Homogeneous seeds of rapeseed (*Brassica napus* L.) cv. Waster treated with two doses of gamma rays viz., 750 Gy and 1000 Gy, two concentrations of EMS i.e., 0.75 %

and 1.00% solution separately and 4 treatments of these mutagenes in combinations (750 Gy+0.75% EMS, 750 Gy + 1.00% EMS, 1000Gy+0.75% EMS and 1000 Gy+1.00% EMS). The treated alongwith untreated (control) seeds were grown in the field with RCB design having 3 replications to raise M_1 generations.

M_2 generations was raised from M_1 seed (5 siliquas from each terminal and primary racemes of each plant were harvested and seed was bulked dose-wise) of each treatment in rows 4 m long and 45 cm apart. Two rows of the control (untreated parent variety) were planted after every 20 rows of treated material to facilitate the comparison during selection. Fifteen M_2 plants were selected at random per treatment to record the observations on yield and yield components. Estimates of mean (x) phenotypic coefficient of variability (C.V.P) genotype coefficient of variability (C.V.g), heritability (h^2) and expected genetic advance (GA) with a selection intensity of 5% were computed according to Larik *et al*., (1980) as under:

$$H^2 (\%) = \delta^2 g / \delta^2 t \times 100$$

where h^2 = Heritability in broad sense, $\delta^2 g$= induced genotypic variance and $\delta^2 t$= Total phenotypic variance calculated from mutagen treated population.

$$GA(\%) = K \times \delta p \times H$$

where, GA= Genetic Advance, K= 2.06 constant for selection differential δp= phenotypic standard deviation of the mean performance of the mutated population and H=Heritability coefficient. The GA was expressed and percentage of the mean for the purpose of comparison.

Results and Discussion

Induced mutations in polygenes governing characters can best be inferred by the estimation of mean, coefficient of variation, heritability and genetic advance in the successive generations of mutagen treated populations. Nature and amount of genetic variability generated in different quantitative traits after exposure to gamma rays and EMS alone and in combination is presented in Tables 1-4.

Plant height was reduced in all the treatments except one i.e., 750 Gy+0.75% EMS. An increase in mutagen doses decreased the plant height by 18.16 and 23.41% in 750Gy and 1000Gy, respectively (Table 1). Reduction in plant height due to gamma rays irradiation has been reported in different crops (Larik *et al*., 1975b; Shah *et al*., 1987, 1990). Short statured plants with other improved characters are very useful because of tolerance to lodging. Highest coefficient of variability (18.51%), heritability (78.24%) and genetic advance (29.84%) for plant height were shown by the mutagen treatments 1000 Gy+0.75% EMS.

Primary branches per plant, in general, were increased in combined treatments and decreased in separate treatments except 1000 Gy (Table 1). This treatment produced the maximum number of primary branches (10.60). Gamma rays increased the number of tillers per plant in wheat (Larik, 1975b) and number of primary branches in rapeseed (Shah *et al*., 1990). The single mutagen treatment 750 Gy produced the highest coefficient of variability (31.77%) and genetic advance (53.33%) while the combined treatment 1000 Gy+0.75%EMS produced the highest heritability (86.00%) (Table 1).

Table 1. Estimates of mean (x), phenotypic coefficient of variation (C.V.p%), genotypic coefficient of variation (C.V.g%), heritability (h^2%) and genetic advance (G.A%) for plant height and primary branches in M_2 generation of Waster.

Treatments	Mean (x)	C.V (p) %	C.V. (g) %	H^2 (%)	G.A % of mean
	Plant Height (cm)				
Controls	178.00	6.71	-	-	
750 Gy	145.66	14.23	11.63	66.78	19.58
1000 Gy	136.33	16.39	13.85	71.43	24.11
0.75% EMS	170.00	9.78	6.81	48.43	9.76
1.00% EMS	139.33	11.64	7.87	45.73	10.96
750 Gy +0.75% EMS	185.67	10.33	8.09	61.24	13.04
750 Gy +1.00% EMS	140.00	14.58	11.82	65.75	19.82
1000 Gy +0.75% EMS	138.33	18.51	16.37	78.24	29.84
1000 Gy +1.00% EMS	131.33	11.42	6.90	36.56	8.60
	Primary branches (Nos)				
Controls	8.60	12.60	-	-	-
750 Gy	7.93	31.77	28.62	81.46	53.33
1000 Gy	10.60	26.64	24.60	85.31	46.75
0.75% EMS	7.53	30.98	27.45	78.47	50.02
1.00% EMS	8.00	31.62	28.59	81.67	53.20
750 Gy +0.75% EMS	10.13	20.98	18.05	74.02	32.06
750 Gy +1.00% EMS	10.13	22.75	20.09	77.93	36.53
1000 Gy +0.75% EMS	10.33	27.98	25.95	86.00	49.56
1000 Gy +1.00% EMS	10.40	25.99	23.83	83.99	44.92

All the mutagen treatments increased the number of siliquas per plant as compared with control (Table 2). An Increase in siliquas per plant after gamma rays treatments has also been reported by other researchers in oleifurous brassica (Chauhan & Kumar, 1986, Naz & Islam. 1979 and Shah *et al.,* 1990). Maximum number of siliquas per plant (723.67) were produced by 750 Gy+ 1.00% EMS. The single treatments produced comparatively less siliquas per plant than combined treatments. The highest coefficient of variation (36.08%) and genetic advance (59.03%) showed in 750 Gy of gamma rays while the maximum heritability (86.82%) was observed in 1.00% EMS. Siliquas per plant are positively correlated with primary branches per plant (Shah *et al*., 1990).

Siliqua length was reduced in all the treatments except in 0.75% EMS (Table 2). The siliqua with minimum length (5.79) was observed in 1000 Gy gamma rays. The combined treatments, in general, reduced less siliqua length than separate treatments of gamma rays and EMS. Mutants with increased siliqua length has also been reported in rapeseed by Shah *et al*., (1990).

All the mutagen treatments reduced the number of grains per siliqua (Table 3). Number of grains per siliqua depends upon siliqua length. With an increase in siliqua length, there is an increase in number of grains per siliqua. The grain per siliqua with minimum length (18.31) was found in the mutagen treatment 1000 Gy of gamma rays. This treatment also produced the highest coefficient of variation (20.91%), heritability (40.89%) and genetic advance (27.56%). The mutants with more number of grains per siliqua than control have been isolated by Shah *et al*., (1990).

Mixed response to mutagens was reported for 1000 grain weight to mutagen treatments (Table 3). The highest 1000-grain weight was recorded in 1000 Gy while the lowest in 750 Gy of gamma rays. Maximum coefficient of variation 44.33%) heritability (85.30%) and genetic advance (71.63%) were recorded in 1.00% EMS. Higher 1000-grain weight is an indication of boldness of the seed. Moreover, the grain weight is negatively correlated with grains per siliqua.

Table 2. Estimates of mean (x), phenotypic coefficient of variation (C.V.p%), genotypic coefficient of variation (C.V.g%), heritability (h^2%) and genetic advance (G.A%) for siliqua per plant and siliqua length in M_2 generation of Waster.

Treatments	Mean (x)	C.V (p) %	C.V. (g) %	H^2 (%)	G.A % of mean
	Siliqua per plant (Nos)				
Controls	394.13	18.92	-	-	
750 Gy	455.60	36 08.	32.16	79.42	59.03
1000 Gy	552.47	31.44	28 .39	81.56	52.82
0.75% EMS	490.40	34 49.	30.96	80.56	57.24
1.00% EMS	638.60	32 17.	29.98	86.82	57.54
750 Gy +0.75% EMS	637.13	24 15.	21.12	76.51	38.06
750 Gy +1.00% EMS	723.67	23 14.	20.72	80.16	38.21
1000 Gy +0.75% EMS	606.33	32 .54	30.13	85.71	57.46
1000 Gy +1.00% EMS	661.33	25.91	23.33	81.06	43.27
	Siliqua length (cm)				
Controls	6.52	13.92	-	-	-
750 Gy	6.17	15.62	5.24	11.25	3.61
1000 Gy	5.79	18.56	9.93	28.65	10.96
0.75% EMS	6.63	14.48	4.22	8.66	2.58
1.00% EMS	6.12	16.12	6.31	15.33	5.11
750 Gy +0.75% EMS	6.13	17.05	8.47	24.66	8.70
750 Gy +1.00% EMS	6.45	17.09	9.70	32.20	11.34
1000 Gy +0.75% EMS	6.30	15.44	5.50	12.75	4.05
1000 Gy +1.00% EMS	6.29	18.52	11.58	39.15	14.92

Table 3. Estimates of mean (x), phenotypic coefficient of variation (C.V.p %), genotypic coefficient of variation (C.V.g%), heritability (h^2%) and genetic advance (G.A%) for grains per siliqua and 1000 grain weight in M_2 generation of Waster.

Treatments	Mean (x)	C.V (p) %	C.V. (g) %	H^2 (%)	G.A % of mean
	Grain per Siliqua (Nos)				
Controls	22.32	20.62	-	-	-
750 Gy	20.73	23.63	8.07	11.66	5.68
1000 Gy	18.31	32.71	20.91	40.89	27.56
0.75% EMS	21.73	22.49	7.35	10.73	4.95
1.00% EMS	17.75	26.93	7.26	7.29	4.03
750 Gy +0.75% EMS	19.84	25.20	9.94	15.49	8.04
750 Gy +1.00% EMS	22.04	23.19	10.07	18.87	9.04
1000 Gy +0.75% EMS	19.95	23.96	6.48	7.31	3.61
1000 Gy +1.00% EMS	21.35	26.08	14.67	31.64	17.00
	1000-grain weight (g)				
Controls	1.39	17.77	-	-	-
750 Gy	1.25	28.24	20.22	51.28	29.58
1000 Gy	2.13	22.36	19.16	72.42	33.62
0.75% EMS	1.35	24.47	16.26	44.14	22.23
1.00% EMS	1.57	44.33	37.82	85.30	71.63
750 Gy +0.75% EMS	1.28	25.38	12.98	26.14	13.46
750 Gy +1.00% EMS	1.64	26.40	21.73	67.41	36.66
1000 Gy +0.75% EMS	1.41	30.78	25.32	67.80	42.59
1000 Gy +1.00% EMS	1.44	24.95	18.11	52.76	27.10

Table 4. Estimates of mean (x), phenotypic coefficient of variation (C.V.p%), genotypic coefficient of variation (C.V.g %), heritability (h^2%) and genetic advance (G.A%) for grain yield per plant and oil (%) in M_2 generation of Waster.

Treatments	Mean (x)	C.V (p) %	C.V. (g) %	H^2 (%)	G.A % of mean
	Grain yield/plant (g)				
Controls	10.50	18.82	-	-	-
750 Gy	10.25	41.91	37.20	78.81	68.03
1000 Gy	11.01	36.94	32.27	76.35	58.14
0.75% EMS	11.85	33.51	29.05	75.19	51.89
1.00% EMS	10.88	38.29	33.71	77.48	61.17
750 Gy +0.75% EMS	11.73	37.29	33.27	77.57	61.06
750 Gy +1.00% EMS	13.74	35.34	32.29	83.43	60.74
1000 Gy +0.75% EMS	11.83	38.50	34.73	81.32	64.44
1000 Gy +1.00% EMS	12.75	41.20	38.18	85.84	72.81
	Oil (%) s				
Controls	39.13	1.89	-	-	-
750 Gy	38.60	3.68	3.09	70.65	5.35
1000 Gy	38.50	3.79	3.22	72.30	5.65
0.75% EMS	38.63	3.93	3.40	74.57	6.04
1.00% EMS	39.04	3.36	2.72	65.70	4.54
750 Gy +0.75% EMS	38.43	3.33	2.65	63.80	4.38
750 Gy +1.00% EMS	38.75	3.66	3.08	70.50	5.31
1000 Gy +0.75% EMS	38.80	3.43	2.79	66.48	4.69
1000 Gy +1.00% EMS	37.63	4.07	3.53	74.89	6.27

Varied response to mutagen treatments was recorded for grain yields per plant (Table 4). The maximum grain yield (13.74) was recorded in 750 Gy +1.00% EMS. The same treatment also produced the highest number of siliquas per plant, primary branches and siliqua length. This is in conformity with Beg (1984), who reported that grain yield in rapeseed and mustard depends upon number of siliquas per plant, number of grains per siliquas and grain weight. The high yielding mutants has been developed from gamma rays irradiated population of rapeseed and mustard (Rehman *et al*., 1987, Shah *et al*., 1999; Thurling & Depittyanan 1992). The treatment 1000 Gy+1.00% EMS showed the highest coefficient of variation (41.20%), heritability (38.18%) and genetic advance (72.81%).

No treatment could produce oil (%) higher than control (Table 4). However, many researchers (Rehman *et al*., 1987, Shah *et al*., 1990, 1999) have isolated the rapeseed mutants with higher oil % than their parents. The highest coefficient of variation (4.07%) and heritability (74.89%) were recorded in mutagen treatment 1000 Gy+1.00%EMS.

It was recorded that all the mutagen treatments shifted the mean values of siliqua per plant in positive direction grains per siliqua and oil (%) in negative direction while the bi-directional response was observed in plant height, primary branches per plant, siliqua length, 1000-grain weight, yield per plant and grain yield per plant. Effect on gamma rays and EMS were more or less equal as separate treatment but higher than the combined treatment showed higher heritability and genetic advance as compared with EMS as separate treatment as well as combined once.

On the basis of overall average, the maximum coefficient of variation was observed for grain yield per plant yield (37.87%) followed by siliquas per plant (29.99%) and thousands grain yield per plant weight (28.36%). The highest heritability values were shown by siliquas per plant (81.78%), primary branches (81.06%) and grain yield (79.49%). The grain yield also showed the highest genetic advance (62.28%) followed by siliqua per plant (50.45%) and primary branches (45.79%) as presented in Table 5.

Table 5. Estimates of mean, phenotypic coefficient of variation (C.V.(p)%), genotypic coefficient of variation (C.V (g)%), heritability (h^2%) and genetic advance (G.A% of mean), for different quantitative traits as affected by mutagens (Average of all the mutagens in M2 generation of Waster).

Genetic parameters	Plant height (cm)	Primary branches (Nos)	Siliquas per plant (Nos)	Siliqua length (cm)	Grains/ siliqua (Nos)	1000 grain weight (g)	Grain yield /plant	Oil (%)
Mean	148.33	9.38	595.69	6.23	20.21	1.50	11.75	38.54
C.V (P)%	13.36	27.33	29.99	6.61	25.52	28.36	37.87	3.65
CV(g)%	10.41	24.64	27.09	7.61	10.59	21.45	38.83	3.06
H^2%	59.56	81.06	81.47	21.58	17.98	58.40	79.49	69.86
GA% of mean	16.96	45.79	50.45	7.66	9.99	34.60	62.28	5.27

The present study provided evidence on the induction of genetic variability in various quantitative characters in rapeseed after treating with gamma rays and EMS alone and in combination. Mutagen derived variability for quantitative characters in crop plants is heritable and response to selection is good (Frey 1969). Thus, genetic variability induced through mutation can be utilized successfully for the evaluation of new varieties of rapeseed with improved agronomic traits.

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(Received for publication 27 October, 2007)