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Fertility improvement in pea (*Pisum sativum* L.) autotetraploids – mutation breeding

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Abstract. C_2 seeds of autotetraploids (colchicine-induced) of two diverse genotypes, T 163 and 5064-S, of pea (*Pisum sativum* L.) and their corresponding diploids were irradiated with 10 kR gamma-ray. Autotetraploids showed a high seed-sterility as compared to diploids. Seed fertility increased in M_2 as compared to M_1. Both quadrivalent and bivalent frequencies decreased in the M_1 generation of autotetraploids in relation to their respective controls. However, a positive shift in the mean bivalent formation was noted at the cost of other configurations in C_3-M_2 with respect to C_2-M_1 and varied with the genotype. In the M_2 generation of autotetraploids, the variability was relatively higher for the number of pods per plant and seed yield per plant. Although the mean values for pods per plant, number of seeds per pod and seed yield per plant remained more or less parallel in autotetraploids in M_1 and M_2 generations, there was an increase in the upper range limit probably due to micromutation for these characters in M_2.

Key words: autotetraploid, disjunction index, fertility improvement, micromutation, *Pisum sativum*, seed set.

Reduced seed fertility is one of the factors, which limits exploitation of autotetraploids, particularly in cereal crops. Hence, studies on the nature and magnitude of fertility and on the ways of its improvement in raw autotetraploids are of considerable importance. Selection for seed set and disjunction index has been shown to improve fertility of raw autotetraploids (KUMAR et al. 1993). The use of mutation breeding – another method of improving autotetraploids – is yet to be explored in pea autotetraploid breeding. Inducing preferential pairing helps to speed up the process of allopolyploidization of autotetraploids

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resulting in the improvement of seed set (SYBENGA 1965, 1966). Induced polygenic variation (micromutation) in raw autotetraploids have been demonstrated after gamma-iradiation in barley (GUPTA, SWAMINATHAN 1967, KUMAR, GUPTA 1973) and safflower (YADAVA 1989). This work analyses the nature and magnitude of induced variation after gamma-irradiation in relation to chromosome behaviour and fertility of autotetraploids of two diverse genotypes of pea (*Pisum sativum* L.).

Material and methods

Air-dried C_2 seeds of autotetraploids of two diverse genotypes, namely, T 163 and 5064-S, and their corresponding diploids were irradiated with 10 kR gamma-ray using Co^{60} source at IARI, New Delhi, India. Fifty seeds from each treatment except diploid control (where 20 seeds were used) were sown in a completely randomized design in the field.

For micromutation studies, seeds of all normal M_1 plants with seed sterility less than 20 per cent in diploid and 55 per cent in autotetraploid were bulked treatment-wise; these bulked seeds were sown in the field in a randomized block design with three replications. Thirty M_2 plants out of 50 were randomly selected from each replication of a treatment for observations on the number of pods per plant, number of seeds per plant, 25-seed weight (g) and seed yield per plant (g). In all these sowings, the row-to-row spacing was 60 cm; plants were spaced 15 cm apart. The crop was fertilized at uniform doses of 20-40-20 N-P-K kg/ha.

A cytological analysis of ten plants taken at random from each treatment was done following KUMAR et al. (1990). Pollen sterility was checked using 2% iodine solution, while seed sterility was estimated by the following relationship:

$$\text{Seed sterility}(\%) = 100 \ \frac{\text{Number of seeds per pod}}{\text{Total number of scares in a pod}} \times 100$$

Results and discussion

The use of mutagens was suggested to bring about improvement in fertility of autotetraploids by inducing preferential chromosome pairing and enhancing variability, probably as a result of micromutation for further selection (MAC KEY 1956, RICHHARIA, GOVINDSWAMI 1963, SYBENGA 1965, 1966, GUPTA, SWAMINATHAN 1967, KUMAR, GUPTA 1973, YADAVA 1989). Since

Table 1. Chromosome behaviour, disjunction index, pollen and seed sterility in diploids (M_1 only) and autotetraploids of two diverse genotypes of pea after gamma-irradiation in M_1 and M_2 generations

Genotype/ Treatment	Chromosome configuration at Metaphase-I per PMC		Disjunction index (%)	Sterility (%)	
	quadrivalent	bivalent		pollen	seed
T 163/5064-S (2x, Control)	Nil	7	100	4.58 (2.2-6.41) 2.50	9.30 (7.2-11.3) 16.13
T 163 (2x, 10 kR, M_1)	0.24 (0.00-0.79 131.91	6.51 (5.38-7.00) 10.14	70.50	36.46 (10.5-66.0) 57.79	30.74 (10.5-50.6) 53.02
5064-S (2x, 10 kR, M_1)	0.05 (0.00-0.42 235.29	6.89 (6.10-7.00) 3.63	75.85	32.98 (9.0-65.0) 67.01	23.52 (11.5-50.6) 88.31
T 163 (4x, Control, C_2)	3.35 (2.55-4.43) 13.43	6.29 (4.32-7.70) 13.35	36.22	24.62 (15-33.1) 23.23	56.34 (54.0-62.4) 7.10
5064-S (4x, Control, C_2)	4.03 (2.60-5.20) 18.11	5.58 (3.16-7.40) 23.67	43.84	23.90 (18.0-35.0) 24.77	47.64 (38.3-56.0) 15.60
T 163 (4x, 10 kR, C_2-M_1)	3.20 (2.43-4.29) 17.50	5.80 (3.86-7.90) 20.69	24.40	39.48 (30.1-48.3) 14.69	61.62 (50.5-70.0) 12.17
5064-S (4x, 10 kR, C_2-M_1)	3.84 (2.55-5.00) 14.84	5.54 (3.60-7.49) 16.97	41.10	26.27 (20.5-29.5) 11.91	54.76 (50.5-58.4) 5.08
T 163 (4x, Control, C_3)	3.35 (2.26-4.95) 21.49	6.31 (3.64-8.41) 23.05	38.25	15.41 (10.0-20.6) 23.86	45.41 (30.1-60.2) 17.93
5064-S, (4x, Control, C_3)	3.73 (2.15-4.84) 21.79	5.94 (4.31-8.43) 24.67	43.00	23.22 (14.4-30.3) 21.68	50.33 (30.1-65.2) 15.81
T 163 (4x, 10 kR, C_3-M_2)	3.24 (2.13-4.80) 18.52	6.42 (4.58-8.60) 11.08	48.50	13.51 (9.0-18.5) 19.32	38.25 (20.2-56.6) 29.78
5064-S (4x, 10 kR, C_3-M_2)	3.76 (2.25-4.86) 17.82	6.08 (5.04-8.91) 11.33	45.00	19.33 (10.5-26.2) 26.84	44.72 (34.4-52.6) 11.78

*Upper, Middle and Lower values relate to the mean, range and coefficient of variation, respectively.

raw autotetraploids are associated with reduced fertility and viability, the optimal dose of gamma-ray (10 kR) was used following BLIXT (1972) and NIGAM and KUMAR (1984) to avoid further toxic effect of the mutagen.

Seed sterility in autotetraploids (Table 1) was high as compared to that of diploids; both pollen and seed sterility were comparable in diploids. As a result of irradiation, seed fertility increased in the C_3-M_2 generations as compared to that of the control (C_3) and in C_2-M_1 of autotetraploids as judged from a decline in sterility values. Similar results were noted by RADU and RAICU (1968) and KUMAR and GUPTA (1973) in barley as well as by RICHHARIA and GOVINDSWAMI (1963) in rice. With regard to chromosome configuration, both quadrivalent and bivalent frequencies decreased in M_1 of autotetraploids as compared to their respective controls. However, in diploids an increase in quadrivalents was associated with a decline in bivalents. Although there was a positive shift in the bivalent frequency mostly at the cost of univalents/trivalents in C_3 over C_2 of the control autotetraploids irrespective of genotype, the magnitude of the shift was not enough to yield remarkable improvement in the seed set. On the other hand, in irradiated populations (C_3-M_2 vs C_2-M_1), a positive shift in the mean bivalent formation was noted at the cost of other chromosomal configurations, particularly in the genotype T 163 as evident from the mean and range. In all these comparisons, the increase in the upper range limit with the decline in the coefficient of variation (CV) for bivalents in C_3-M_2 appeared to be remarkable leading to an increase in seed fertility, i.e. seed set. Such positive shift in the bivalent frequency was also noted in autotetraploids of *Dactylis* (STEBBINS 1956), *Brassica campestris* (SRINIVASCHAR, SINGH 1967) and maize (SEMENOV et al. 1973).

The analysis of variance revealed highly significant treatment differences at the both ploidy levels in M_2 for the traits. A positive shift in the mean seed fertility in autotetraploids of the both genotypes in C_3-M_2 as compared to C_2-M_1 (Table 1) was encouraging and was also reflected in the increase of the seed number per pod in M_2 plants as related to the control (Table 2). Although mean values for the number of pods per plant, number of seeds per pod and seed yield per plant in M_2 did not differ significantly as judged from the critical difference value (CD), the increase in the upper range limit, indicating induction of micromutation, was encouraging, particularly in autotetraploids. Further, the magnitude of induced variability, as judged from CV, was comparable at the both ploidy levels; it was relatively larger (1.5- to 2-fold) in the latter (Table 2) for the number of pods per plant and seed yield per plant. A similar trait-specific variability after irradiation of autotetraploids was noted by RICH-

Table 2. The mean, range and variation coefficient of seed yield and yield traits in autotetraploids (C_2), their corresponding diploids and controls in two diverse genotypes of pea in M_2-C_3 generation after gamma-irradiation*

Parameter	2x control		4x control		2x, 10 kR		4x, 10 kR	
	T 163	5064-S	T 163	5064-S	T 163	5064-S	T 163	5064-S
Number of pods per plant								
Mean	32.57	29.57	19.53	23.35	25.98 (–6.59)	24.23 (–5.34)	20.20 (0.67)	21.97 (–1.38(
Range	25-38	23-40	13-32	18-31	10-45	17-40	8-40	10-46
C.V.	10.22	11.53	19.87	12.76	24.71	20.26	31.63	33.41
Critical difference at 5% = 1.64								
Number of seeds per pod								
Mean	3.67	3.62	1.48	2.43	3.21 (–0.46)	3.21 (–0.41)	1.63 (0.15)	2.48 (0.05)
Range	2.4-5.5	2.5-4.8	0.9-2.2	1.2-3.9	1.4-5.1	1.7-4.8	1.0-2.8	1.0-4.0
C.V.	19.51	15.47	24.32	22.22	30.84	23.05	22.70	29.84
Critical difference at 5% = 0.17								
25-seed weight (g)								
Mean	4.52	3.61	6.79	4.87	4.68 (0.16)	3.43 (–0.18)	6.66 (–0.13)	4.70 (–0.13)
Range	4.0-5.2	3.1-4.0	5.1-8.9	4.3-5.5	4.0-5.3	3.0-3.9	5.0-8.2	4.0-5.6
C.V.	6.64	7.20	15.17	7.39	7.05	7.87	15.16	9.70
Critical difference at 5% = 0.27								
Seed yield per plant (g)								
Mean	11.63	9.61	4.24	5.93	9.04 (–1.79)	9.08 (–0.53)	4.92 (0.68)	5.27 (–0.66)
Range	9.5-14.5	8.6-10.2	3.6-6.0	5.1-6.8	5.5-14.8	6.1-14.5	2.3-13.3	2.1-14.8
C.V.	9.89	4.44	15.33	7.77	20.63	23.46	46.95	51.23
Critical difference at 5% = 0.85								

*Values given in the parentheses relate to a shift in the means of M_2 generation from their respective control.

HARIA and GOVINDSWAMI (1963), GUPTA and SWAMINATHAN (1967) and KUMAR and GUPTA (1973).

A few tetraploid M_2 selections recorded increased mean values for all the traits except the number of seeds per pod in the both genotypes as compared to their respective controls. Shifts in the means from their respective diploid controls for the number of pods per plant, 25-seed weight and seed yield per plant in autotetraploids were positive; the shift was larger in the genotype 5064-S than in T 163.

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