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Gamma Ray- and EMS-Induced Bold-Seeded High-Yielding Mutants in Chickpea (*Cicer arietinum* L.)

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Abstract: Three bold-seeded high-yielding mutants were isolated from the M_2 progeny of chickpea var. Pusa-212, viz. Pusa-212 A (200 Gy), Pusa-212 C (400 Gy), and Pusa-212 F (300 Gy + 0.2% EMS). Data on various quantitative traits, such as plant height, number of branches per plant, number of pods per plant, number of seeds per pod, 100-seed weight (g), seed yield (g) per plant, and protein content (%), were recorded for all 3 mutants in the M3 generation. The mutants were morphologically quite distinct, as compared to the control and to each other. The most notable change was in seed size and shape of these mutants, giving them bold characteristics as compared to small seeds in the control. Mean performance of different quantitative traits was significantly better among the mutants as compared to the control, with a few exceptions. Seed protein content did not show any significant positive correlation with seed size and test weight. Heritability estimates were quite high for almost all polygenic traits among the mutants, in comparison to the control. Cytological analysis of the mutants was almost normal, except for a few chromosomal aberrations.

Key Words: *Cicer arietinum* L., EMS, gamma rays, macro mutations, quantitative traits, heritability

Gama Işınları ve EMS Nohutta (*Cicer arietinum* L.) Koyu Tohumlu Yüksek Verimli Mutantları Uyarmıştır

Özet: Üç adet koyu tohumlu yüksek verimli mutant, nohutun var. Pusa-212 M_2 soylarından [Pusa-212 A (200 Gy), Pusa-212 C (400 Gy) ve Pusa 212 F (300 Gy + %0.2 EMS)] izole edilmiştir. Bitki yüksekliği, her bitkinin dal sayısı, tohum zarflarının sayısı, her tohum zarfındaki tohum sayısı, 100 adet tohumun ağırlığı (g), her bitkinin tohum miktarı ve % protein içeriğindeki çeşitli nicel özelliklerdeki veriler, M3 neslindeki üç mutant için kaydedilmiştir. Mutantlar, kontrol ile karşılaştırıldıklarında, onlardan oldukça farklıydı. Kontrol grubundaki küçük tohumlar ile karşılaştırıldığında, en fark edilebilir değişiklik, tohum büyüklüğünde ve onları koyu karakteristik yapan bu mutantların şeklindeydi. Farklı nicel özelliklerin ortalama performansı, kontrol ile karşılaştırıldığında birkaç istisna hariç, mutantlar arasında oldukça yüksekti. Tohum protein içeriğinin yüzde oranı, tohum büyüklüğü ve test ağırlığı ile önemli bir pozitif ilişki göstermemiştir. Kalıtımsal tahminler, hemen hemen bütün filogenetik özellikler için, kontrole nazaran mutantlar arasında oldukça yüksekti. Mutantların sitolojik analizleri, birkaç kromozomal bozukluk hariç, hemen hemen normaldi.

Anahtar Sözcükler: *Cicer arietinum* L., EMS, gama ışınları, makro mutasyonlar, nicel özellikler, kalıtımsal

Introduction

Chickpea (*Cicer arietinum* L.) is the most important grain legume crop of the Indian subcontinent. Conventional approaches to plant breeding have exploited the available genetic variability in the chickpea, which has in turn led to a narrow genetic base for this crop. Mutation breeding is an important method used for the improvement of crops through the induction of mutations at loci that control economically important traits and/or by eliminating undesirable genes from elite breeding lines (1). It has been demonstrated by several researchers that genetic variability for several desired characters can be successfully induced through mutations and its practical value in plant improvement programs has been well established. Seed mutagenesis has been used for enhancing genetic variability of yield parameters (2-5) and the induction of chlorophyll mutations (6-8), early flowering (9), male sterility (10), herbicide tolerance (11), and morphological mutations (12-16). In addition

to the vital role in plant improvement programs, a new role for induced mutations in releasing gene silencing in transgenic plants has been reported (17). The main advantage of mutation breeding is the potential for improving one or a few characters without changing the rest of the genotype. Success, however, depends on controlling and directing the induced mutation process for the production of desirable mutations. Keeping this in mind, an attempt was made to induce genotypic alterations to enhance the genetic variability and improve the yield potential of chickpea through the use of physical and chemical mutagens. The present study deals with some bold-seeded high-yielding mutants isolated in the M_2 and M_3 generations of mutagenized chickpea populations.

Materials and Methods

Certified seeds of chickpea (*Cicer arietinum* L.) var. Puse-212 were procured from the Genetics Division, IARI, New Delhi. Dry and healthy seeds (10%-12% moisture content) were treated with different doses of gamma rays (100, 200, 300 and 400 Gy) from a ^{60}Co source at the National Botanical Research Institute, Lucknow, and EMS (0.1%, 0.2%, 0.3%, and 0.4%) for 6 h. A set of irradiated seeds was also used for combination treatment with EMS (200 Gy + 0.2% EMS, 200 Gy + 0.3% EMS, 300 Gy + 0.2% EMS, and 300 Gy + 0.3% EMS) for 6 h after pre-soaking them in distilled water for 12 h. Thereafter, the treated and control seeds were sown in the field (3 replicates) in a complete randomized block design (CRBD) in order to raise the M_1 generation. Each treatment/dose consisted of 300 seeds, including the control. The seed-to-seed and row-to-row distance was maintained at 15 cm and 20 cm, respectively. Seeds from M_1 plants were harvested separately. For raising the M_2 generation, 50 M_1 plants and 30 seeds from each plant were selected for each treatment, including the control, and were planted in the plant progeny rows (3 replicates) in a complete randomized block design. Several mutants for growth habit, leaf shape, floral morphology, and pod and seed shape and size were isolated from mutagenized populations. Three bold-seeded high-yielding mutants, viz. Pusa-212 A, Pusa-212 C, and Pusa-212 F, were isolated, respectively, from 200 Gy, 400 Gy, and 200 Gy + 0.2% EMS M_2 progeny. A set of 75 seeds from each mutant type was sown the following year (3 replicates of 25 seeds each) to raise the M_3 generation, with seed-to-seed and row-to-row distances the same as above. Data for various quantitative traits were recorded from 30 randomly selected plants (10 in each replicate) of each mutant type, as well as the control. Statistical analysis of various quantitative traits was performed as per standard statistical procedures (18). Broad-sense heritability (h^2) was estimated by the formula suggested by Johnson et al. (19). For meiotic analysis young flower buds were collected from 5-10 randomly selected M_3 plants and fixed in a 1:3 solution of acetic acid and ethyl alcohol, supplemented with a few crystals of ferric chloride, for 24 h. The buds were then stored in 70% ethyl alcohol in a refrigerator. Slides were prepared using 2% acetocarmine, using the standard squash technique. Seed protein content of the mutants was evaluated using the method of Lowry et al. (20).

Results and Discussion

As yield per plant is the most desirable character in any breeding program, certain mutants that were distinctly superior to untreated populations, with regard to seed yield per plant, were isolated in the M_2 generation (Table 1, Figure 1-3). These mutants were morphologically quite distinct, especially with regard to seed size and shape, as compared to the control.

Table 1. Brief description of the bold-seeded mutants isolated in *Cicer arietinum* L. var. Pusa-212.

Mutant	Treatment	Salient features
Pusa-212	Control	Seeds semi-round, small, and shrunken
Pusa-212 A	200 Gy gamma rays	Round, bold-seeded and high-yielding
Pusa-212 C	400 Gy gamma rays	Giant, bold-seeded and high-yielding
Pusa-212 F	300 Gy + 0.2% EMS	Bold, rough seed coat, and high-yielding

Figure 1a,b. Pods and seeds of bold- and round-seeded mutant Pusa-212 A.

Figure 2a,b. Pods and seeds of giant mutant Pusa-212 C.

Figure 3a,b. Pods and seeds of bold- and rough-seeded mutant Pusa-212 F.
Note: Control pods and seeds in all the figures are on left side

The bold-seeded mutant (Pusa-212 A) isolated from 200 Gy M_2 progeny showed an increase in the size of leaflets, flowers, pods, and seeds. Plants were upright and straight, with light green foliage, as compared to the dark green foliage of the control. The seeds were bold, round, and smooth, with a bright yellow seed coat. Pusa-212 C isolated from 400 Gy M_2 progeny showed gigas-like characteristics and vigorous growth. The plant remained initially straight, but subsequently exhibited a trailing habit due to heavy secondary branching. The leaves, stipules, flowers, pods, and seeds were almost double the size as those of the control. Pusa-212 F isolated from the combination treatment of gamma rays and EMS (300 Gy + 0.2% EMS) was tall, with dark green foliage; the seeds were dark yellow with a rough and hard seed coat. All these mutants were confirmed as true

breeding in the M_3 generation. Gamma ray-induced bold-seeded mutants have also been reported in faba bean (15), *Vigna mungo* L. (16), chickpea (21), and linseed (22).

Mean, shift in mean, and heritability (h^2) estimates for various quantitative traits in the mutants, in comparison to the control, are presented in Table 2. Plant height and the number of primary branches per plant increased significantly ($P \geq 0.01$, 0.05) in all 3 mutants. Maximum plant height (62.82 ± 1.06) and number of primary branches per plant (8.33 ± 0.49) were recorded in Pusa-212 F. The number of pods increased considerably in Pusa-212 A (163.40 ± 6.05) and Pusa-212 F (176.13 ± 5.22), as compared to 144.53 ± 1.98 in the control. In contrast, Pusa-212 C showed a significant decrease in pods per plant, but a considerable increase in seed weight (31.73 ± 0.59 g) compared to the control (12.64 ± 0.14 g), resulting in an overall increase in seed yield per plant of the mutant (38.86 ± 1.69 g), as compared to the control (30.05 ± 0.59 g). A similarly significant increase in seed weight and seed yield per plant ($P \geq 0.01$) was also recorded in Pusa-212 A and Pusa-212 F. Mean seeds per pod was not significantly affected in the present investigation. The bold-seeded mutants demonstrated multiple mutations affecting many traits, particularly those of leaves and seeds. Such bold-seeded mutants could be attributed to mutation of the pleiotropic gene, mutation of gene clusters, or to chromosomal rearrangement. Increased seed yield following increases in leaf, flower, pod, and seed size has been reported in other pulse crops (23,24).

It is evident from Table 2 that the increase in mean seed yield per plant was due to an increase in the mean performance of other yield-contributing traits, including pods per plant, seed size, and test weight. All the mutant populations showed a high degree of heritability of almost all quantitative traits studied, as compared to the control. This could have been due to the fact that the M_3 plants of each mutant type belonged to the same parent plant that was isolated in the M_2 generation, so that the chances of increased homozygosity of alleles was greater in such plants, as compared to the control, which was a random sample of seeds selected from M_2 control plants. This increased heritability, especially for yield-contributing

Table 2. Mean ($\bar{X}$), shift in mean, and heritability (h^2) estimates of various quantitative characters in the bold-seeded mutants isolated in *Cicer arietinum* L. var. Pusa-212.

Mutant Type	Plant height (cm)	Number of primary branches per plant	Number of pods per per plant	Number of seeds per pod	100-seed weight (g)	seed yield per plant (g)	Seed protein content (%)
Pusa-212 Control							
Mean	59.21 ± 0.71	5.73 ± 0.28	144.53 ± 1.98	1.58 ± 0.09	12.64 ± 0.14	30.05 ± 059	
Shift in mean	0.00	0.00	0.00	0.00	0.00	0.00	20.19 ± 0.03
h^2 (%)	41.52	41.68	38.29	47.22	44.54	37.49	
Pusa-212 A							
Mean	63.02 ± 0.88	6.40 ± 0.43	163.40 ± 6.05	1.59 ± 0.04	22.03 ± 0.23	44.12 ± 1.22	
Shift in mean	+3.81*	+0.67	+18.87*	0.01	+9.39*	+14.07*	19.56 ± 0.55
h^2 (%)	53.85	57.17	51.31	72.73	86.56	57.12	
Pusa–212 C							
Mean	61.66 ± 0.85	7.46 ± 0.42	124.33 ± 4.06	1.35 ± 0.03	31.73 ± 0.59	38.86 ± 1.69	
Shift in mean	+2.45*	+1.73*	–20.20*	–0.23*	+19.09*	+8.81*	19.20 ± 0.53
h^2 (%)	48.67	63.32	61.72	54.38	72.43	52.72	
Pusa-212 F							
Mean	62.82 ± 1.06	8.33 ± 0.49	176.13 ± 5.22	1.68 ± 0.04	22.65 ± 0.20	46.42 ± 1.13	
Shift in mean	+3.61*	+2.60*	+31.60*	0.10	10.01*	+16.37*	20.5 ± 0.22
h^2 (%)	65.12	60.57	59.50	81.35	75.70	66.42	

h^2: Broad-sense heritability ($\sigma^2g/\sigma^2p \times 100$), where σ^2g = genotypic variance and σ^2p = phenotypic variance.
*Significant at 1%.

Table 3. Frequency of meiotic aberrations in the isolated mutants of *Cicer arietinum* L. var. Pusa-212.

Mutant Type	No. of PMCs scored	Total frequency of abnormal PMCs (%)			Total abnormalities (%)
		Metaphase	Anaphase	Telophase	
Control	296	0.67	1.01	0.34	2.02
Pusa-212 A	309	1.62	0.97	1.29	3.88
Pusa-212 C	346	1.16	1.73	0.87	3.76
Pusa-212 F	324	1.23	1.54	1.85	4.62

traits, may lead to quick stability of these mutants. Seed protein content of the mutants was not altered to a greater extent than that of the control, suggesting a very low positive or negative correlation with seed size. Similar results were also reported by Kharkwal (25).

The bold-seeded mutants isolated in the present investigation are of special interest, as these mutants showed considerable improvement in yield, as well as increased pod size. Cytological observations of these mutants revealed 8 bivalents (2n = 16) at the metaphase. Anaphase and telophase segregations were normal, although some meiotic aberrations, such as stickiness, precocious separation, and univalent formation, were also observed (Table 3). The maximum frequency of abnormal PMCs was recorded in the mutant Pusa-212 F (4.62%), followed by Pusa-212 A (3.88%), and Pusa-212 B (3.76%), versus 2.02% in the control, suggesting the absence of any major structural changes at different loci in these mutants. The normal cytological behavior of these mutants might indicate their genetic nature; however, cryptic structural changes in the chromosomes cannot be denied. As the mutants were isolated in the M_2 generation, they are expected to be recessive in nature. Sjodin (26) characterized mutations in *Vicia faba* as gene mutations because there were no visible chromosomal changes associated with them. The present bold-seeded mutants also fall into this category, as no major changes were observed in their chromosomal structure or meiotic behavior, with a few exceptions. These mutants may be utilized in various breeding programs as donor parents for the boldness character of the mutant or, alternatively, they could be released as new cultivars after extensive analysis in future generations.

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References

1. Lippert LF, Berg BO, Cook AA. Three variegated seedlings in the pepper. J Hered 55: 78-93, 1964.
2. Jabeen N, Mirza B. Ethyl methane sulphonate enhances genetic variability in *Capsicum annuum*. Asian J Pl Sci 1: 425-428, 2002.
3. Singh G, Sareen PK, Saharan RP et al. Induced variability in mungbean (*Vigna radiata* (L) Wilczek). Indian J Genet 61: 281-282, 2001.
4. Sharma D. Induced variability following single, combined and recurrent doses of EMS and gamma rays in green gram (*Vigna radiata* (L.) Wilczek). J. Nuclear Agric Biol 27: 35-43, 1998.
5. Ignacimuthu S, Babu CR. Induced quantitative variation in wild and cultivated urd and mungbean. J Nuclear Agric Biol 22: 133-137, 1993.
6. Waghmare VN, Mehra RB. Induced chlorophyll mutations, mutagenic effectiveness and efficiency in *Lathyrus sativus* L. Indian J Genet 61: 53-56, 2001.
7. Kharkwal MC. Induced mutations in chickpea (*Cicer arietinum* L.) II. Frequency and spectrum of chlorophyll mutations. Indian J Genet 58: 465-474, 1998.
8. Sarkar A, Sharma B. Frequency and spectrum of chlorophyll mutations in lentil (*Lens culinaris* Medik.). Thi J Agri Sci 22: 107-111, 1989.
9. Thurling N, Depittayanan V. EMS induction of early flowering mutants in spring rape (*Brassica napus*). Plant Breeding 108: 177-184, 1992.

10. Maan SS, Williams ND. An EMS induced dominant allele for male sterility transferred euplasmic wheat. Crop Sci 24: 851-852, 1984.

11. Sebastian SA, Fader GM, Unlrich JF et al. Semi dominant soybean mutations for resistance to sulphoryl urea herbicides. Crop Sci 24: 851-852, 1989.

12. Sangsiri C, Sorajjapinum W, Srinives P. Gamma radiation induced mutations in Mungbean. Science Asia 31: 251-255, 2005.

13. Lyakh VA, Lagron VA. Induced mutation variability in *Linum grandiflorum* Desp Mut Breed Newsl 1: 5-6, 2005.

14. Muthusamy A, Vasanth K, Jayabalan N. Induced high yielding mutants in cotton (*Gossypium hirsutum* L.). Mut Breed Newsl 1: 6-8, 2005.

15. Joshi P, Verma RC. Radiation induced pod and seed mutant in faba bean (*Vicia faba* L.). Indian J Genet 64: 155-156, 2004.

16. Singh RK. Gamma rays induced bold seeded mutant in *Vigna mungo* (L.) Hepper. Indian J Genet 56: 104-108, 1996.

17. Bhatia CR. Release of gene silencing in transgenics: A new role for induced mutations. Mut Breed. Newsl., 44: 3-5, 1999.

18. Singh RK, Chaudhary BD. Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publications, New Delhi;1977, 47.

19. Johnson HW, Robinson HF, Comstock RE. Estimates of genetic and environmental variability in Soybean. Agron J 47: 314-318, 1955.

20. Lowry OM, Rosenbourgh NJ, Farr AL et al. Protein measurement with folin phenol reagent. J Biol Chem 193: 265-275, 1951.

21. Gumber RK, Singh S, Singh K. Frequency and spectrum of mutations induced by gamma rays in Desi and Kabuli Chickpea. Int. Chickpea Newsletter 2: 8, 1995.

22. Badre RS, Choudary AD. Induced mutations in Linseed (*Linum usitatissimum* L.). Indian J Genet 64: 159-160, 2004.

23. Swaminathan MS. Basic research need to further improvement of pulse crops.In.South East Asia Nutritional Improvement of Food Legume by Breeding. Proc. Symp. Protein Advisory Group UN, 3-5 July, (1972) Rome: 61-68, 1973.

24. Prasad MVR. Induced mutations in green gram. Indian J Genet 36: 218-222, 1976.

25. Kharkwal MC. Induced mutations for improvement of protein in chickpea (*Cicer arietinum* L.). Indian J Genet 58: 61-68, 1998.

26. Sjodin J. Induced morphological variation in *Vicia faba* L. Hereditas 155-180, 1971.