International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 24-09-2021, Accepted: 08-10-2021, Published: 25-10-2021
Volume 6, Issue 5, 2021, Page No. 1449-1451

Improving bold seed mutant in chickpea using induced mutagenesis

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Abstract
In present investigation well known chemical mutagens (sodium azide and EMS) and a physical mutagen (gamma radiation) were employed to induce genetic variability for improvement of chickpea cultivar 'Vishwas' (Phule G-5). Seeds were treated with three different concentrations/doses of SA (2, 3 and 4 mM), EMS (8, 12 and 16 mM) and gamma radiation (400, 500 and 600 Gy). M_1 and M_2 to M_5 generation were raised for isolation and characterization of mutants. SA, EMS and gamma radiation induced leaf, pod, seed, flower colour and morphological mutants. Bold seed mutant was isolated in M_2 generation from 2 mM SA treatment. True breeding behavior of bold seed mutant was studied for various quantitative and qualitative traits in M_3 to M_5 generation. Induced mutant lines showed both positive and negative increases in quantitative traits. True breeding mutant lines in the M_4 and M_5 generation differed considerably in their quantitative traits from the control. The bold seed mutant showed significantly increased for 100 seed weight and yield per plant over the control. The yield and yield contributing traits and protein content and protein profile were studied during the M_5 generation. The mutant had the increased protein content as compared to control. The proteins bands differ from control seeds, control seed exhibited 12 bands where as mutant with 11 bands.

Keywords: chickpea, mutagenesis, protein, SA

Introduction
Chickpea (*Cicer arietinum* L.) is an important self-pollinated grain legume. Hybridization, spontaneous and induced mutations are three main sources of variation for chickpea breeding. Chickpea is cultivated on about 10 million ha worldwide with a yield of 8.28 million tons annually (Salimath 2007) [9]. Chickpea is recognized as major source of protein in human diet. However, its yield did not witness much appreciation during past decade (Barshile *et al.* 2006) [2], due to the lack of genetic variability (Wani and Anis 2001) [16], limited genome plasticity and self-pollination (Singh 2009) [11]. Increasing world population and their consumption requirements are placing extraordinary demands on agriculture (Sandeepani 2021) [10]. A common and efficient tool to create genetic variability in chickpea is induced mutagenesis (Micke 1988) [7]. The efficiency of induced mutagenesis to generate mutations valuable for plant breeders has been widely proven and documented through the official release of 3,364 mutant varieties (IAEA, 2021) [4]. The developed varieties improve biodiversity and serves as a baseline for conventional plant breeding thus directly contributing to the conservation and use of plant genetic resource. During present investigation, an attempt was made for improvement of the locally adapted cultivar 'Vishwas', through induced mutagenesis employing potent mutagens like gamma radiation, sodium azide and ethyl methane sulphonate for quantitative and qualitative improvement.

Materials and Methods
Seeds of Chickpea (Cicer arietinum L.) cultivar Viswas (Phule G-5) were procured from the Mahatma Phule Krishi Vidyapeeth, Rahuri, India. Healthy seeds containing 10-12% water were treated separately with chemical (SA and EMS) and physical (gamma radiation) mutagens. For chemical mutagen treatments, seeds were presoaked in distilled water for 6 hours and then subjected to 2, 3 and 4 mM SA and 8, 12 and 16 mM EMS, for 12 hours at 25±2°C. Treated seeds were thoroughly washed under running tap water for an hour to terminate the reaction of the chemical. For physical mutagen treatment, dry seeds were irradiated with 400, 500 and 600 Gy from a ^{60}Co source available in the Department of Biophysics, Government Institute of Science, Aurangabad (M.S., India). Each treatment was carried out for 250 seeds. All treated seeds along with control were sown in the field at a spacing of 15cm within rows and 45cm between rows to raise the M_1 generation. All M_1 plants were harvested separately to raise M_2 generation. The M_2 progeny was raised following randomized block design with 3 replications. Each treatment comprised of 20-21 M_1 plant progenies and each M_2 progeny row consisted of 10 to 25 plants in three replications. The cultural operations and application of FYM were done as per the standard schedule. The M_2 progeny was screened for early mutations. Frequency of early mutations was scored throughout the life span of the M_2 progeny. Mutation frequency was calculated as the percentage of mutated plants. True breeding behaviour and characterization of an early mutant was carried out. Data collected in M_3 to M_5 generations were analyzed statistically. Standard error (SE) was calculated following by Snedecor and Cochran (1967)[14]. Data were collected on 8 agronomic traits *viz.*, plant height (cm), plant spread (cm), number of pods/plant,

number of seeds/plant, yield/plant (g), 100-seed weight (g), protein content and days of maturity. Total proteins were estimated following the method of Lowry *et al*. (1951) [6]. The analysis and comparison of proteins profile were carried out by the SDS-PAGE following the method of Laemmli (1967) [5].

Results and Discussion

All treatments of mutagens were not effective in inducing bold seed mutations at M_2 generation, which were otherwise completely absent in the control, EMS and gamma radiation and lower concentration of SA. The frequency of bold seed mutations varied with concentration and the mutagen. High frequency of bold seed mutations was observed with SA treatments. The frequency of bold seed mutations ranged from 0.18 to 0.40 (Table 1). 4 mM SA treatment induced maximum frequency of bold seed mutations. On the basis of percentage of frequency of mutations, it can be concluded that the SA was most potent in inducing bold seed mutations in M_2 generation than EMS and gamma radiation.

Table 1: Frequency of induced bold seed mutations in M_2 progeny of chickpea cultivar Vishwas.

Treatment Conc./Dose	M_2 population	Bold seed mutation frequency
Control	574	00
SA 2mM	532	00
SA 3mM	548	0.18
SA 4mM	495	0.40
EMS 8 mM	518	00
EMS 12 mM	573	00
EMS 16 mM	712	00
GR 400 Gy	513	00
GR 500 Gy	535	00
GR 600 Gy	564	00

Bold seed mutant was compared and evaluated for mean values of quantitative traits with parental cultivar Vishwas in the M_3 to M_5 generation. Both positive and negative variation was found in the quantitative traits as compared to parental cultivar (Table 2). The bold seed mutant showed a significant increase in the 100 seed weight in M_5 generation over the control. Bold seeded mutants in this variety exhibited increase in yield per plant as compared to the control. The proteins content of the mutant and the control do not have remarkable differences. Bold seed mutant was very similar to those of control plants in characters like plant height, plant spread, number of seeds per plant etc.

Table 2: Characterization of bold seed mutant in M_3 to M_5 generation of chickpea.

Quantitative traits	Control	Bold seed mutant		
		M_3 generation	M_4 generation	M_5 generation
	Mean SE	Mean SE	Mean SE	Mean SE
Plant height (cm)	35.5 ± 0.29	34.13±0.31	34.10±0.38	35.21 ± 0.29
Plant spread (cm)	24.82 ± 0.22	28.66±0.31	27.73±0.65	27.93 ± 0.67
Number of pods/plant	24.82 ± 0.4	24.66±0.42	25.89±0.46	25.83 ± 0.28
Number of seeds/plant	26.65 ± 0.74	26.9±0.63	26.78±0.68	26.91 ± 0.82
Seed yield/plant (gm)	7.42 ± 0.10	9.09±0.27	9.22±0.17	9.53 ± 0.19
100-seeds weight (gm)	28.15 ± 0.32	37.75±0.65	36.83±0.27	37.81 ± 0.31
Protein content (%)	26.4 ± 0.29	28.58±0.32	26.6±6.71	27.51 ± 0.32
Days of maturity	108.7 ± 1.25	110.0±1.22	110.58±1.73	109.6 ± 1.23

Bold seeded mutants have been reported earlier by Wani and Anis (2001) [16], Gumber *et al*., (1995) [3], Thomas *et al*., (1990) [15] in chickpea and Pawar (2001) [8] in black gram. Singh (1996) [13] has also reported similar mutants in *Vigna*. Singh *et al*., (2000) [12] isolated a bold seeded mutant in urdbean following mutagenesis with gamma rays and EMS. The bold seeded mutants may be utilized in various breeding programmes as donor parent for boldness character (Wani and Anis, 2001) [16]. Pawar (2001) [8] has successfully used bold seeded mutants with higher 100-seed weight in cross breeding programmes.

A marked difference was observed in presence or absence of some polypeptide bands in the mutants, as compared to control. Control plants exhibited 12 polypeptide bands. Bold seeded mutant exhibited a total 11 bands. This mutant was characterized by the absence of bands 1, 4 and 7, which were found in control, and presence of two additional bands (bands 12 and 13) with molecular weights 20.89 and 18.20 kDa which were otherwise not found in control (Table 3 and fig.1).

Table 3: Protein profile of early mutant of chickpea cultivar Vishwas in M_5 generation.

Control / Mutants	Number of bands and molecular weight (kDa)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	93.33	63.10	55.59	51.29	46.77	41.21	34.37	30.20	26.92	24.55	21.38	20.89	18.20	16.6
Control	+	+	+	+	+	+	+	+	+	+	+	-	-	+
Bold seed mutant	-	+	+	-	+	+	-	+	+	+	+	+	+	+

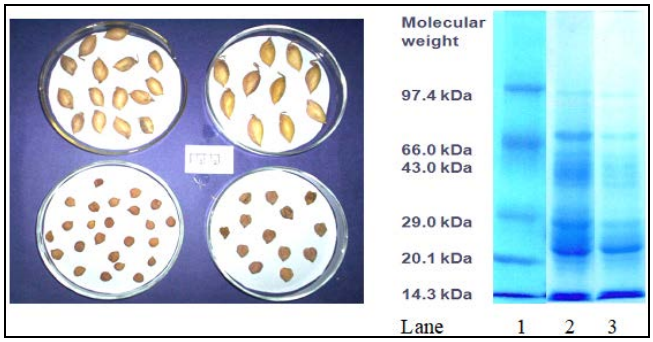

Fig 1: Control (a), bold seed mutant (b) and SDS-PAGE protein profile (left lane 1 Molecular marker, lane 2 control and lane 3 bold seed mutant) in M_5 generation.

On the basis of percentage of frequency of mutations, it can be concluded that the SA was most potent in inducing bold seed mutations in M_2 generation than EMS and gamma radiation. SA was observed as a potent mutagen for induction of bold seed mutant in chickpea. The present studies on the electrophoretic banding pattern of seed protein could provide quick identification of genetic variability useful for genetic improvement of chickpea.

Conclusion

Bold seeded mutant was isolated from treatment of 3mM and 4 mM SA in the variety Vishwas. It was similar to control in most of the morphological traits. The number of seeds per pod and 100 seed weight were significantly increased over the control. SA is more effective than EMS and gamma radiation for induction of the bold seed mutant.

Acknowledgements

The authors are thankful to Mahatma Phule Krishi Vidyapeeth for providing the seeds sample and Dr. S. B. Pawar, Principal, Shri Anand College, Pathardi (M.S.) for providing the necessary laboratory facilities.

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