



CHARACTERIZATION OF BOLD SEED MUTANT IN CHICKPEA CULTIVAR

VIJAY

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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 'Vijay' (Phule G 81-1-1). 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). M₁ and M₂ to M₅ generation were raised for isolation and characterization of mutants. SA, EMS and gamma radiation induced leaf, pod, seed, flower colour and morphological mutants in chickpea. Bold seed mutant was isolated in M₂ generation from 3 mM SA treatment. True breeding behavior of bold seed mutant was studied for various quantitative and qualitative traits in M₃ to M₅ generation. Induced mutant lines showed both positive and negative increases in quantitative traits. True breeding mutant lines in M₅ generation differed considerably in their quantitative traits from the control. The bold seed mutant showed significantly increased for yield per plant and 100 seed weight over the control. The yield and yield contributing traits and protein content and protein profile were studied during the M₅ generation. The mutant had the increased protein content as compared to control. The proteins bands differ from control seeds, control seed exhibited 13 bands where as mutant with 14 bands.

Keywords: Chickpea, Electrophoresis, Mutagenesis, SA, Protein.

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 second most cultivated grain legume in the world after *Phaseolus* bean. Chickpea is cultivated on about 10 million ha worldwide with a yield of 8.28 million tons annually (Salimath 2007). 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), due to the lack of genetic variability (Wani and Anis 2001), limited genome plasticity and self-pollination (Singh 2009). A common and efficient tool to create genetic variability in chickpea is induced mutagenesis (Micke 1988). During present investigation, an attempt was made for improvement of the locally adapted cultivar 'Vijay', 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 Vijay (Phule G 81-1-1) were procured from the Mahatma Phule Krishi Vidyapeeth, Rahuri, India. Uniform and 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 ⁶⁰Co 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 M₁ generation. All M₁ plants were harvested separately to raise M₂ generation. The M₂ progeny was raised following randomized block design with 3 replications. Each treatment comprised of 20-21 M₁ plant progenies and each M₂ 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 M₂ progeny was screened for bold seed mutations. Frequency of bold seed mutations was scored throughout the life span of the M₂ progeny. Mutation frequency was calculated as the percentage of mutated plants. True breeding behaviour and characterization of bold seed mutant was carried out. Data collected in M₃ to M₅ generations were analyzed statistically. Standard error (SE) was calculated following by Snedecor and Cochran (1967). 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). The analysis and comparison of proteins profile were carried out by the SDS-PAGE following the method of Laemmli (1967).

RESULT & DISCUSSION :

All treatments of mutagens were not effective in inducing bold seed mutations at M₂ 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 0.17 to 0.32 (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 M₂ generation than EMS and gamma radiation.

Bold seed mutant was compared and evaluated for mean values of quantitative traits with parental cultivar Vijay in the M₃ to M₅ 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 M₅ 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.

A marked difference was observed in presence of some polypeptide bands in the mutants, as compared to control. Control plants exhibited 13 polypeptide bands. Bold seeded mutant exhibited a total 14 bands. This mutant was characterized by presence of one additional band (band 3) with molecular weight 59.57 kDa which were otherwise not found in control (Table 3 and fig.1).

Bold seeded mutants have been reported earlier by Wani and Anis (2001), Gumber *et al.*, (1995), Thomas *et al.*, (1990) in chickpea and Pawar (2001) in black gram. Singh (1996) has also reported similar mutants in *Vigna*. Singh *et al.*, (2000) 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 (Vani and Anis, 2001). Pawar (2001) has successfully used bold seeded mutants with higher 100-seed weight in cross breeding programmes.

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 M₅ 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 M₂ 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.

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Table 1. Frequency of induced bold seed mutations in M₂ progeny of chickpea cultivar Vijay

Treatment Conc./Dose	M ₂ population	Bold seed mutation frequency
Control	625	00
SA 2mM	431	00
SA 3mM	582	0.17
SA 4mM	615	0.32
EMS 8 mM	525	00
EMS 12 mM	541	00
EMS 16 mM	550	00
GR 400 Gy	596	00
GR 500 Gy	539	00
GR 600 Gy	519	00

Table 2. Characterization of bold seed mutant in M₃ to M₅ generation of chickpea cultivar Vijay

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)	35.7 ± 0.28	34.13±0.31	33.15±0.31	34.92 ± 0.31
Plant spread(cm)	23.63 ± 0.31	28.66±0.21	26.18±0.46	27.14 ± 0.53
Number of pods /plant	25.01 ± 0.37	24.66±0.42	28.16±0.38	26.37 ± 0.32
Number of seeds /plant	26.7 ± 0.63	29.9±0.56	30.5±0.58	27.8 ± 0.71
Seed yield/plant (gm)	7.65 ± 0.15	9.09±0.26	8.9±0.18	8.79 ± 0.21
100-seeds weight (gm)	28.93 ± 0.36	37.75±0.45	34.93 ±0.35	35.12 ± 0.41
Protein content (%)	26.43 ± 0.28	28.53±0.38	28.8± 0.71	28.65 ± 0.41
Days of maturity	108.7 ± 1.84	110.0±1.26	107.0 ± 1.8	108.0 ± 1.29

Table 3. Protein profile of early mutant of chickpea cultivar Vijay in M₅ generation

Control / Mutants	Number of bands and molecular weight (kDa)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	95.5	63.1	59.57	54.35	48.98	45.19	39.36	31.26	28.51	24.83	21.63	19.05	14.45	13.8
Control	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Bold seed mutant	+	+	+	+	+	+	+	+	+	+	+	+	+	+

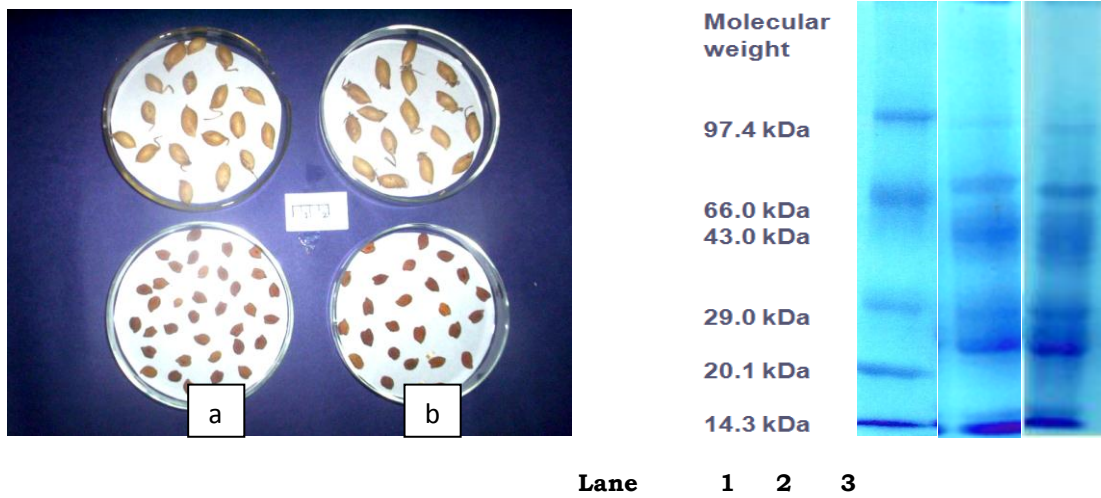


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