CHEMICAL MUTAGENESIS INDUCED SMALL LEAF MUTANT IN CHICKPEA (*CICER ARIETINUM* L.)

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Abstract

Chickpea (*Cicer arietinum* L.) is most important pulse crop among the legumes. It is rich in protein. In spite of its importance it has narrow genetic diversity. Morphological mutations affecting almost all the parts of the plant and seed characteristics were isolated in M₂ generation of a popularly grown cultivar of chickpea, Phule G-81-1-1 (Vijay), treated with chemical mutagens, sodium azide (SA) and ethyl methane sulphonate (EMS). They included leaf, pod, seed, flower colour and morphological mutants. A high frequency of viable mutations was observed with EMS followed by SA treatments. The small leaf mutant with better canopy was isolated in M₂ generation from 12 mM EMS treatment. Breeding behavior of this mutant was studied for various quantitative and qualitative traits in M₅ generation. True breeding mutant lines in the M₅ generation differed considerably in their quantitative traits from the control. Induced mutant lines showed both positive and negative increases in quantitative traits. The yield and yield contributing traits and protein content were studied during the M₅ generation. The mutant showed the increased, plant spread, number of pods per plant and number of seeds per plants. The protein content and protein profile showed variation as compared to control.

Keywords: chickpea, EMS, mutagenesis, SA.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crop. It is a valuable source of dietary protein. In pulses especially in chickpea genetic variability has been exhausted due to natural selection (Wani and Anis, 2001). Its yield did not witness much appreciation during the past decade. It has been argued that one of the reasons for failure to achieve break through in productivity in self-pollinated crops like chickpea as compared to the cereals is the lack of sufficient genetic variability. Therefore, development of new plant types for different situations is required. Mutation techniques can be deployed for creating and enlarging the genetically conditioned variability of a species within a short time. (Barshile *et al.*, 2006). Genetic variability is the most essential prerequisite for any successful crop improvement programme as it provides a spectrum of variants for an effective selection process (Jahagirdar *et al.*, 2005). In the present study, an attempt has been made to induce genetic variability for developing novel mutants in the chickpea cultivar Vijay through induced chemical mutagenesis.

Material and Methods

Seeds of the chickpea cultivar, Vijay (Phule G-81-1-1) used in the present study were procured from Mahatma Phule Krishi Vidyapeeth, Rahuri (Maharashtra). To begin with, pilot experiments were conducted to determine the lethal dose (LD₅₀), suitable

concentrations of the mutagens and duration of treatment of chickpea. From such experiments it was finally established that concentrations of 2, 3 and 4 mM SA and 8, 12 and 16 mM EMS for duration of 12 hours are best suitable for mutagenic treatments. The seeds were soaked in distilled water for 6 hours prior to treatment with different concentrations of chemical mutagens. SA solutions were prepared in 1 M phosphate buffer (pH 3.2), while EMS solutions were prepared in 0.1 M phosphate buffer (pH 7) at 25 ± 2°C with intermediate shaking. The volume of mutagenic solutions was about 5 times as that of seeds. The seeds treated with chemical mutagens were thoroughly washed under running tap water for an hour to terminate the reaction of the chemical before sowing. Each treatment was carried out for 250 seeds. All treated seeds along with control were sown in the field at spacing of 15 cm in rows and 45 cm between rows to raise M₁ generation. For raising M₂ generation, the M₁ plants were harvested separately and seeds sown in a randomized block design (RBD) with 3 replications at experiment field of Shri Anand College, Pathardi. The frequency and spectrum of mutations were studied in M₂ generation. Small leaf true breeding mutant maintained up to M₅ generations were studied and evaluated using RBD with 3 replications. Data collected in M₅ generation were analyzed statistically. Standard error (SE) was calculated following by Snedecor and Cochran (1967). Data were collected on 10 agronomic traits viz., plant

height (cm), plant spread (cm), number of pods/plant, number of seeds/plant, yield/plant(g), 100-seed weight (g), leaf area, biological yield, harvest index and protein content. 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 (1970).

Results and Discussion

Spectrum and frequency of mutations: Spectrum and frequency of mutations in M₂ generation was comprised of 66 families with a total plant population of 3244 surviving plants at harvest. The morphological mutants isolated mainly showed an altered leaf structure, plant shape, seed size, seed colour, seed structure and days of the maturity. A high frequency and spectrum of viable mutations was observed in the M₂ generation of chickpea cultivar Vijay with both mutagens, which were completely absent in the control, and increased in a concentration/dose dependent manner of the mutagen employed (Table 1).

A high frequency of viable mutations was observed with EMS followed by SA treatments. The frequency of viable mutations ranged from 1.15 to 5.34. 16 mM EMS treatment induced a high frequency of leaf morphological mutations (2.40). On the other hand, SA treatments showed the least spectrum and lowest frequency of viable mutations. Kharkwal (2000) attributed the differences in frequency and spectrum of viable mutations induced by various mutagens to genetic differences in the cultivars, while Konzak, *et al.*, (1961) have reported that even as small as a single gene difference could bring about significant changes not only in the spectrum but also the frequency of recoverable mutations. In this research with chickpea cultivar Vijay, we observed that the spectrum and frequency of induced viable mutations increased with increasing concentrations of SA

and EMS. This could be due to a differential mode of action of the mutagens on different base sequences in various genes.

Small leaf mutant was compared and evaluated for mean values of quantitative traits with parental cultivar 'Vijay' in the M₅ generation. Both positive and negative variation was found in the quantitative traits as compared to parental cultivar (Table 2). The small leaf mutant showed a significant increase in the plant spread, number of pods per plant, number of seeds per plant and seed yield per plant and significantly reduced leaf area in M₅ generation over the control (Fig. 1). The proteins content of the mutant and the control do not have remarkable differences (Table 2).

The small leaf mutants showed significant increase in plant height, plant spread, number of pods per plant, number of seeds per plant and significantly reduced leaf area. Similar mutants were reported earlier in chickpea by Kharkwal (2000) and in red gram by Khan and Veeraswamy (1974). According to Tarar and Dnyansagar (1979) changes in leaf size and shape are due to chromosomal aberrations produced in the plant as a result of action of chemical mutagens and ionizing radiations. Thakur and Sethi (1993) reported that the small leaf character is governed by a single recessive gene and can be used in the development of composite crosses and in breeding methods.

Protein banding pattern revealed variation in number of bands and molecular weights in the small leaf mutant. SDS polyacrylamide gel electrophoresis, for proteins profile exhibited 12 bands for control whereas small leaf mutant, showed 13 protein bands. The present studies on the electrophoretic banding pattern of seed protein could provide quick identification of genetic variability useful for genetic improvement of chickpea.

Table 1. Spectrum and frequency of mutation in chickpea cultivar Vijay in M₂ generation.

Mutagenic treatment	Plant population	M ₁ plant families	Leaf mutants	Plant type mutants	Pod /seed mutants	Sterile mutants	Total frequency of mutations
Control	300	21	00	00	00	00	00
2 mM SA	431	21	00	0.92	0.23	00	1.15
3mM SA	582	21	00	1.03	0.51	00	1.54
4mM SA	615	21	0.16	1.47	0.65	00	2.28
Mean			0.16	1.47	0.65	00	2.28
8 mM EMS	525	21	1.33	1.71	0.57	00	3.62
12 mM EMS	541	21	1.08	1.80	0.73	0.36	3.97
16 mM EMS	550	21	2.40	1.65	1.10	0.18	5.34
Mean			1.60	1.72	0.80	0.18	4.31

Table 2. Mean performance for quantitative traits between the control (parental cultivar) and the Small leaf mutant in M₅ generation of chickpea cultivar Vijay.

Quantitative traits	Control	Small leaf mutant
	Mean SE	Mean SE
Plant height (cm)	30.05 ± 0.32	32.67 ± 0.25
Plant spread (cm)	24.62 ± 0.42	30.63 ± 0.67
Number of pods/plant	35.79 ± 0.3	72.23 ± 0.59
Seeds /plant	36.56 ± 0.94	75.31 ± 1.76
Seed yield /plant (gm)	8.74 ± 0.12	12.02 ± 0.25
100-seeds weight (gm)	23.65 ± 0.47	13.01 ± 0.61
Leaf area (cm ²)	5.00 ± 0.23	3.31 ± 0.21
Biological yield (gm /plant)	13.90 ± 0.25	12.73 ± 0.17
Harvest index (%)	64.77 ± 0.8	58.04 ± 1.53
Protein content (%)	25.53 ± 0.54	26.16 ± 0.49
Days of maturity	101.11 ± 1.23	107.18 ± 1.2

Figure 1. Field view of mutant and control in M₅ generation and SDS-PAGE protein profile of cultivar Vijay and small leaf mutant (lane 1 molecular marker, lane 2 small leaf mutant and lane 3 control).**Acknowledgements**

I am thankful to Mahatma Phule Krishi Vidyapeeth for providing the germplasms and Dr. B.J. Apparao for rendering necessary guidance. I am also thankful Dr. S. B. Pawar, Principal, Shri Anand College, Pathardi for providing the necessary laboratory facilities.

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