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INDUCED MUTATIONS FOR IMPROVEMENT OF PROTEIN IN CHICKPEA (*CICER ARIETINUM* L.)

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ABSTRACT

Variability has been induced and screened for crude protein content in fortyfour chickpea micro-mutants and thirtyfive macro-mutants. A wide range of variability has been generated through treatments of physical (gamma rays and fast neutrons) and chemical mutagens (EMS and NMU) and mutant genotypes having significantly higher protein content than parent variety as well as standard check have been isolated. The results of the present study suggest that it is possible to increase the protein content concurrent with an increase or without a loss in grain weight, density and yield through induction of mutations.

Key words: Chickpea, induced mutations, micro-mutant, macro-mutant, protein, variability, grain weight, grain density

Grain legumes are important and rich sources of protein in human and animal nutrition. They contain 20-30% protein in their seed, which is 2 to 3 times higher than that in the cereals. The protein of pulses is nutritionally superior and important as the amino acid lysine is found in larger quantity than in the cereal protein. This becomes particularly important in a cereal-pulse diet [1]. Chickpea is the most important grain legume crop accounting for the largest area and production of all pulse crops. It is consumed in several preparations all over the world predominantly by the vegetarian population. Chickpea is not only rich in total protein (above 20%) but it has the highest Protein Efficiency Ratio (PER) among grain legumes. However, due to rapid spread and availability of high yielding and input responsive cereal crop varieties, chickpea has faced a tough competition in recent years. Inspite of intensive breeding efforts, chickpea yields have not shown any appreciable increase during the past decade leading to sharp increase in its price and reduction in per capita availability. Improvement of total protein content in the existing high yielding chickpea cultivars could be one of the possible methods of achieving the minimum requirement of 60 g per capita per day of protein [2] of the ever increasing protein malnourished vegetarian population. One of the reliable and less time consuming techniques for improving the protein content in crop plants is through induced mutagenesis [3-12]. Results obtained in the present study further confirm the utility of induced mutations for improvement of protein content in chickpea.

MATERIAL AND METHODS

The experimental material for the present study comprised of two distinct sets of induced mutants - a) micro-mutants and b) macro-mutants. The first set consisted of forty-four micro-mutants (twenty-four belonging to *desi* type, twelve *kabuli* type and eight green-seeded type) and six control, each type having two control varieties, one parent and one standard check. The second set consisted of thirty-five macro-mutants (seventeen *desi*, ten *kabuli* and eight green-seeded types) and four parent varieties of chickpea and one wild species *Cicer judaicum*. Micro- and macro-mutants used under present study were developed from four varieties of chickpea, two *desi* (var. G 130 and var. H 214) and two culinary (one *kabuli* C 104 and one green-seeded var. L 345) treated with different doses of gamma rays (40, 50 and 60 Krad), fast neutrons (0.5, 1.0 and 1.5 Krad), N-nitroso-N-methyl urea (NMU) [0.01% (20h) and 0.02% (8h)] and ethyl methane sulphonate (EMS) [0.1% (20h) and 0.2% (8h)]. The treatment of chemical mutagens NMU and EMS for 8 hours was given after 12 hours of presoaking while that of 20 hours was without presoaking. Five hundred seeds were used in each treatment. Micro-mutants were isolated in M_2 and M_3 generations through a new selection technique [12] for efficient screening of useful induced variability. The *desi* micro-mutant entries studied for protein estimation also included the three high yielding and diseases resistant mutant varieties Pusa 408 (Ajay), Pusa 413 (Atul) and Pusa 417 (Girnar), released for commercial cultivation in India [12]. Thirty-five viable macro-mutants affecting different morphological characters such as growth habit, foliage morphology and seed characters were also selected to study seed protein content along with their respective parents as controls and also a wild species - *Cicer judaicum*. Crude protein content in the genotypes was estimated by standard micro-Kjeldahl method [13] using two replications. Grain weight and density observations were recorded for each genotype. Grain density was worked out by dividing the weight of the grain by its volume, which was determined by using Hexane (liquid) displacement [14].

RESULTS AND DISCUSSION

A. INDUCED VARIABILITY FOR PROTEIN CONTENT IN MICRO-MUTANTS

Analyses of micro-mutants revealed that a highly significant variability for protein content has been induced. Crude protein contant of chickpea genotypes ranged from 18.82 to 27.99%. *Desi* chickpea showed a wider range compared to *kabuli* and green-seeded types (Table 1). Highly significant differences for protein content and 100 grain weight between and within the groups of the *desi, kabuli* and green-seeded chickpea genotyes were observed (Table 2).

Table 1. Range, mean and coefficient of variation for protein content, grain weight and density in *desi, kabuli* and green-seeded chickpea micro-mutants

Group	Range	Mean	CV(%)
	Protein content (%)		
desi	18.820 - 27.700	24.138 ± 0.290	2.03
kabuli	21.350 - 27.450	24.481 ± 0.307	1.98
green	21.710 - 27.990	25.763 ± 0.385	1.75
pooled	18.820 - 27.990	24.501 ± 0.199	2.02
	100 grain weight (g)		
desi	11.556 - 13.950	12.652 ± 0.070	2.86
kabuli	20.130 - 28.312	22.919 ± 0.467	1.78
green	12.710 - 20.304	15.658 ± 0.596	2.41
pooled	11.556 - 28.312	16.128 ± 0.475	2.41
	grain density (g/cc)		
desi	1.185 - 1.395	1.276 ± 0.006	1.48
kabuli	1.295 - 1.450	1.358 ± 0.008	0.40
green	1.265 - 1.350	1.309 ± 0.006	0.47
pooled	1.185 - 1.450	1.305 ± 0.005	1.08

Table 2. ANOVA for protein content, grain weight and density in chickpea micro-mutants

Source	df	protein content ms	100 grain weight ms	grain density ms
Between Groups	2	20.013**	962.02**	0.055
Within Groups	47	7.205**	6.49**	0.005

The mean protein content among the mutants (Table 3) varied from 19.05 to 27.76% as compared to 22.47 to 25.48% in parents and standard controls. The overall mean of protein content in the selected mutants was higher than the control means. Out of 44 mutants, 33 showed significantly higher protein content ranging from 1.30 to 18.88% than the respective controls. In general, the mutants of *desi* type showed relatively higher increase in protein content over their best control than the culinary type mutants. Among the 24 mutants of *desi* type, ten mutants showed more than 10% increase over the parent var. G 130. In case of culinary mutants, 9 out of 12 *kabuli* and 6 out of 8 green-seeded mutants had significantly higher protein content over their best checks respectively.

The results of this study make it amply clear that by using an efficient selection technique, a significant improvement in protein content of the desi, kabuli and green-seeded chickpea genotypes can be achieved through induced mutagenesis. This technique is based on the principle that from a purely practical breeding point of view, the M2 families of greater interest are only those for which the CV for yield and various other yield component characters studied has increased and, or has not greatly altered over the control. In view of their high yield potential expressed in advanced generation evaluations, most of these mutant cultures were tested under the All India Coordinated Trials as entries named under BGM series and showed promising yield performance in various trials and zones for more than two to three years. It has been possible to isolate mutant genotypes in which the percentage of protein has increased with or without an increase in grain weight and grain density (Table 3). Protein content was found to have a low positive correlation with grain weight ($r = 0.039$) and grain density ($r = 0.115$). However, grain weight showed a significantly positive correlation ($r = 0.647$) with grain density which has been reported to be one of the important characters to be used as selection criterion for grain yield [14]. This observation indicates that, the protein content can be increased with an increase in grain density along with increase or with minimal adverse influence on grain weight. Another interesting observation from the present analysis is that the high protein mutants do not necessarily suffer from a loss in grain yield as is evident from the fact that the three desi chickpea mutant varieties, Pusa 408, Pusa 413 and Pusa 417, out of these micro-mutants showing significant improvement in protein percentage have already been released in India for higher productivity and disease resistance. Such micro-mutant genotypes can obviously be expected to be of practical value in a protein improvement programme and would offer a greater potentiality in being used for cross breeding since their increased protein contant is not expected to be at the expense of grain yield, grain density or grain weight. Hyprosola (M-699) a gamma ray induced mutant of chickpea var. Faridpur-1 developed and released in Bangladesh [15] with 4% higher protein and 20% superiority in grain yield over its parent is another practical example to support the above findings.

Table 3. Protein content, grain weight and grian density in chickpea micro-mutants, parents and controls

Variety	Protein content (%)	Grain weight (g)	Grain density (g/cc)	Variety	Protein content (%)	Grain weight (g)	Grain density (g/cc)
	desi type				***kabuli* type**		
G 130 (Parent)	23.035@	13.01@	1.383@	C104 (Parent)	22.925	22.00	1.339
C 235 (St. Ch.)	22.470	11.98	1.352	L550(St. Ch.)	23.410@	22.66@	1.347@
BGM 405	23.335*	13.00	1.310	BGM 415	24.920*	22.03	1.349
Pusa 408 (Ajay)$	25.065*	13.20*	1.286	BGM 424	21.355	22.94*	1.326
Pusa 413 (Atul)$	25.175*	12.68	1.248	BGM 429	22.068	20.43	1.333
BGM 416	25.810*	12.50	1.340	BGM 430	24.535*	20.23	1.409*
Pusa 417 (Girnar)$	26.315*	12.65	1.248	BGM 441	24.865*	22.83*	1.312
BGM 418	25.010*	12.33	1.255	BGM 442	24.645*	21.19	1.411*
BGM 419	25.830*	12.19	1.248	BGM 445	22.765	27.79*	1.331
BGM 421	26.000*	12.45	1.365	BGM 447	25.525*	20.24	1.345
BGM 425	23.750*	12.79	1.227	BGM 462	25.085*	20.79	1.362*
BGM 426	25.270*	11.75	1.208	K 56	24.355*	25.30*	1.330
BGM 427	22.155	2.70	1.270	K 114	27.015*	26.20*	1.380*
BGM 428	26.436*	12.83	1.243	G 40	26.480*	26.24*	1.435*
BGM 431	21.790	12.77	1.257				
BGM 432	21.070	12.53	1.245		**green-seeded type**		
BGM 433	20.335	13.87*	1.262	L345 (Parent)	25.190	12.88	1.292
BGM 435	25.895*	12.25	1.280	Hima(St. Ch.)	25.480@	13.72@	1.303@
BGM 436	25.030*	13.29*	1.271	BGM 452	21.915	13.85	1.279
BGM 437	26.050*	12.80	1.255	BGM 453	26.970*	13.26	1.313*
BGM 438	27.380*	12.69	1.241	BGM 455	26.810*	14.10*	1.331*
BGM 439	24.225*	12.60	1.281	BGM 456	23.995	20.13*	1.277
BGM 440	19.055	11.85	1.274	BGM 463	26.285*	14.08*	1.320*
BGM 443	22.790	12.79	1.281	BGM 464	27.055*	19.21*	1.336*
BGM 444	23.340	12.02	1.265	BGM 475	26.160	18.50*	1.325*
BGM 450	24.885*	12.91	1.283	BGM 476	27.765*	16.75*	1.314*
CD at 0.05 % =	0.198	0.156	0.006	* significant increase over check			

@ = Best check compared
$ = Released mutant varieties of chickpea

Table 4. Induced variability for protein content in macro-mutants in chickpea

Mutant	Variety & Treatment	Protein (%)	Mutant	Variety & Treatment	Protein (%)
	***desi* var. G 130**			***kabuli* var. C 104**	
Close pinnae-1	NMU 0.01% (20h)	28.82*	Upright mutant	NMU 0.01% (20 h)	27.12*
Narrow leaf	NMU 0.01% (20h)	28.58*	Round grains	neutrons 1.0 Krad	26.90*
Miniature	EMS 0.1% (20h)	28.40*	Spreading mutant	EMS 0.2% (8h)	26.68*
Mosaic grains	EMS 0.2% (8h)	25.99*	Dwarf & early	EMS 0.01% (20h)	25.37*
Close pinnae-2	r-rays 50 Krad	24.90*	Bouquet	EMS 0.2% (8H)	25.16*
Tall mutant	NMU 0.02% (8h)	24.68*	Long grains	neutrons 0.5 Krad	25.16*
Tiny leaf	NMU 0.02% (8h)	24.02*	Bold grains	NMU 0.01% (20h)	23.62*
Small leaf	EMS 0.1% (20 h)	22.22	Fasciata	NMU 0.01% (20h)	23.41*
G 130 (Parent var.)	Control	23.15	Round smooth grains	EMS 0.2% (8h)	22.75
			Dwarf bushy	EMS 0.2% (8h)	20.78
	***desi* var. H 214**		C 104 (Parent var.)	Control	22.84
Smooth grains	neutrons 1.0 Krad	27.75*		**Green seeded var. L 345**	
Bold grains	neutrons 1.0 Krad	27.12*	Lobbed pinnae	r-rays 40 Krad	28.40*
Brownish grains	neutrons 1.0 Krad	26.68*	Compact-1	NMU 0.02% (8h)	28.18*
Round green grains	neutrons 1.5 Krad	26.68*	Miniature	NMU 0.01% (20h)	26.52*
Round red grains	r-rays 60 Krad	24.95*	Gigas-1	NMU 0.02% (8h)	25.34
Gigas	NMU 0.02% (8h)	24.28*	Compact-2	NMU 0.02% (8h)	25.12
Dark red grains	neutrons 1.0 Krad	23.19	Super gigas	NMU 0.01% (20 h)	23.59
Round white grains	neutrons 1.0 Krad	22.75	Spreading	r-rays 50 Krad	23.20
Bold brown grains	neutrons 1.0 Krad	21.88	Stalked leaflet	r-rays 40 Krad	20.74
H 214 (Parent var.)	Control	23.62	L 345 (Parent var.)	Control	25.07

GM = 25.05 CV = 2.51% CD at 5% = 0.283 *Cicer judaicum* (wild species) 26.42*

B. VARIABILITY FOR PROTEIN CONTENT IN MACRO-MUTANTS

A group of 35 macro-mutants, isolated in early generations due to their distinct morphological features were also analysed for protein content. The protein content among the macro-mutants (Table 4) varied from 20.74 to 28.82% as against the range of 22.84 to 25.07% in the controls. Highly significant induced variability (MS = 0.352**) was observed among the macro-mutant genotypes studied. Out of 35 mutants, 24 showed significantly higher protein content than the respective controls. Among the 17 mutants of *desi* varieties, 13 had significantly higher protein content and the increase ranged from 2.79 to 23.18% over the controls. The widest range and increase in protein content was seen in *desi* var. G 130. In case of *kabuli* var. C 104, eight mutants out of ten had significantly higher protein. Green-seeded var. L 345 showed lowest number and degree of increase in protein content in its mutants. In general, the *desi* varieties appeared to have relatively more total seed protein than the *kabuli* and the green-seeded types. Interestingly the protein content of 26.42% in the wild species *Cicer judaicum* was also well above the general mean of the mutants and control varieties.

In view of the several reports on the induction of variability for improvement in the protein content in cereals: wheat [3-5]; rice [6]; barley [7-8]; maize [9-10]; sorghum [11] and other legume crops [12, 14-18], one important approach to solve the problem of protein malnutrition of the vast majority of vegetarian population would be to increase the quantum of protein in pulse crops as well as its quality especially in respect of essential amino acids in which the Indian diet as a whole is defficient. The results of this study clearly indicate that useful variability for quality characters like protein content can be sucessfully induced, isolated and significantly improved through mutagenesis in grain legume crops. This conclusion is evident from the fact that a number of induced mutant varieties with improved protein content, particularly in case of grain legumes have been developed and reported by several workers [12, 14-18].

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