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INDUCED GENETIC VARIABILITY IN CHICKPEA (*CICER ARIETINUM L.*) III. FREQUENCY OF MORPHOLOGICAL MUTATIONS

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Abstract

A comparative study for frequency of morphological mutations induced by physical (gamma rays) and chemical (EMS) mutagens in M_2 population was conducted in two desi (Pb2000, C44), one each of kabuli (Pb1) and desi x kabuli introgression genotypes (CH 40/91) of chickpea. In M_2 population, about 63 different types of morphological mutations in different parts of the plants, such as branching pattern, stem structure, growth habit, foliage type, plant height, pod and seed size, foliage color, flowering behavior and maturity was observed. The highest frequency of morphological mutations was observed in 300 Gy gamma irradiation (4.11%) of CH40/91 followed by 0.4% EMS (2.08%) of desi genotype C44. The overall frequency of mutations in physical mutagen was high in CH40/91 (2.69%) followed by Pb2000 (1.38%), C44 (1.02%) and Pb-1 (0.27%). The overall frequency of morphological mutations in chemical mutagen was very low in Pb2000 (0.45%) followed by Pb-1 (0.78%), C44 (1.53%) and CH40/91 (1.90%) as compared to physical mutagens. Overall frequency of morphological mutations in M_2 population also indicated maximum mutability (2.30%) in introgression genotype (CH40/91) followed by desi genotype C44 (1.28%) and Pb2000 (0.91%), whereas kabuli type had lowest mutation rate (0.52%). The significant higher morphological as well as chlorophyll frequency of CH40/91 as compared to other three genotypes could be due to the diverse genetic nature. The gamma rays and EMS treatments differ in their mutation spectra/frequency.

Introduction

Chickpea (*Cicer arietinum* L.) is a diploid with 2n=2x=16 (Arumuganathan & Earle, 1991) and is a highly self-pollinated crop with an outcrossing rate of less than 1%. It is an essential source of protein (25%) in human diet and plays a key function in the richness of soil by fixing atmospheric nitrogen through symbiotic process. The available genetic variability in the chickpea has been exploited through conventional plant breeding approaches which have in turn led to a narrow genetic base for this crop. Induced mutation is another vital tool used for the improvement of crops through the introduction of mutations at loci that organize economically important traits and/or by removing undesirable genes from elite breeding lines (Lippert *et al.*, 1964)). Several researchers have recognized that genetic variability for desirable traits can be effectively induced through mutations (Gaur & Gour, 1999; Atta *et al.*, 2003; Nayyar *et al.*, 2005; Ganapathy *et. al.*, 2008) and its practical significance in plant improvement programmes has been well recognized. Morphological mutations affecting different plant parts can be of enormous practical utility and many of them have been released directly as crop varieties (Shah *et al.*, 2010).

Creating genetic variations has become increasingly important as crop genetic resources are becoming more difficult to be obtained *via* plant exploration and other programme(s). Hence, the present investigations were taken up to study the phenotypic as well as genotypic alterations induced through the application of physical and chemical mutagen(s). A complete stabilization of the induced changes was achieved through the selection of eliminations in subsequent generations. These characteristics induced holds promise for further exploitation in plant breeding programmes.

In the present investigation all the segregants in the M_2 generation of four commercially adapted desi, kabuli and desi x kabuli chickpea genotypes (Pb2000, C44, Pb-1 and CH40/91), resulting from mutagenic seed treatment of gamma irradiation and EMS were studied.

Materials and Methods

Genetic variability was induced in two desi (Pb2000 and C44), one kabuli (Pb-1) and one desi x kabuli recombinant genotype (CH40/91) through gamma irradiation (200, 300, 400, 500, and 600 Gy) and ethyl methane sulphonate (EMS) (0.2%, 0.3% and 0.4%). The radio sensitivity, LD_{50} and doses of physical and chemical treatments were determined (Shah *et al*., 2008). The material for the present study comprised of individually harvested M_1 plants during winter 2000-2001. All the M_1 plants were harvested for raising M_2 generation. The number of surviving plants in C44 and Pb-1 were quite large (a range of 251 to 412 plants in C44 and 345 to 668 plants in Pb-1 respectively) in all the treatments. The number of surviving plants in Pb2000 and CH40/91 were less than 250 (except for 300 and 400Gy in Pb2000 and only 400Gy treatments in CH40/91, respectively). Seed samples from each plant were advanced based on M_1 plant-to-row method in the field at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad from mid-October 2001 to April 2002. The respective non-treated control was also planted after every tenth row for comparison with normal variation.

In each row 20 plants at 15 cm apart were grown. The distance between row to row was 30cm. The experimental soil was slightly sandy (pH= 7.56), rich in organic matter and high in N content. At the time of “Rauni (Pre-sowing irrigation)”, field was treated with Biflex (Termiticide) @ 2.5 liter ha^{-1} to protect crop from the attack of termites. 125 kg ha^{-1} of DAP fertilizer was applied prior to planting. The experiment was not given any irrigation except seasonal rainfall. The weeding was performed manually three times during cropping season. All deviating plant types (growth habits, leaf types, stem types, flower types, flower colors, seed types and seed colors mutants) were observed and recorded in M_2 throughout the cropping season. M_2 plants showing a difference from the normal plants with desired phenotypes were harvested individually. The mutation frequency was computed on M_2 family basis (% of mutated progenies) and M_2 plant basis (% mutants) after confirming the true breeding behavior of M_2 variants as described by Kharkwal (1999).

Single and multiple mutations: In addition to large number of single morphological mutations, multiple mutations affecting two or more characters were found common in all the mutagenic treatments in the present study. Most of the families segregating for one type of mutation are called as Type I. The families segregating for two or three types of morphological mutations and called as Type II and Type III mutations respectively.

Results and Discussion

The frequency of morphological mutations recorded in terms of segregating families and mutant plants in the M_2 is depicted in Table 1. Differences were noticed in the mutation rates obtained among four genotypes in the treatments of gamma rays and EMS. Of the two mutagens, the highest frequency of viable mutations in M_1 progeny was shown by 300 Gy gamma ray (23.78%) followed by 0.4% EMS (15.52%). In M_2 population, the highest frequency of morphological mutations showed that 300 Gy gamma irradiation in CH40/91 was most efficient (4.11%) followed by 0.4% EMS (2.08%) in desi genotype C44. The overall frequency of mutations in chemical mutagens was very low in Pb2000 (0.45%) followed by Pb-1 (0.78%), C44 (1.53%) and CH40/91 (1.90%) as compared to physical mutagens (Table 2). The overall frequency of morphological mutations (Table 2) in physical mutagens was high in CH40/91 (2.69%) followed by Pb2000 (1.38%), C44 (1.02%) and Pb-1 (0.27%). Overall frequency of viable morphological mutations on M_2 population also indicated maximum mutability (2.30%) in introgression genotype (CH40/91) followed by desi genotype C44 (1.28%) and Pb2000 (0.91%), whereas kabuli type had lowest mutation rate (0.52%). It appears that induced mutability is also governed by the architecture of the material used. The kabuli type genotype (Pb-1) exhibited (Table 2) overall least morphological mutation frequency in gamma irradiation (0.27%). The present studies support the findings of Kharkwal (1999) and Toker & Cagirgan, (2004) who observed kabuli genotype as most sensitive to mutagens. It is observed that the genetic background of the chickpea variety has a significant role in determining its mutability. The evidence of genetic differences as small as single gene difference could bring about significant changes not only in the frequency but also in the spectrum of recoverable mutations have been provided by other workers (Gustafsson, 1947; Varughese & Swaminathan, 1968).

Table 1. Frequency of morphological (macro) mutants in M_2 generation of four chickpea genotypes.

Genotypes	Treatments	Frequency on M_1 progeny basis			Frequency on M_2 population basis		
		Total progenies	**Segregating progenies**	**%**	**Total plants**	**Mutants**	**%**
Pb. 2000	300Gy	254	29	11.42	3756	63	1.68
	400Gy	295	29	9.83	4254	46	1.08
	0.3%EMS	202	11	5.45	3587	18	0.50
	0.4% EMS	184	8	4.35	3319	13	0.39
	Overall			**7.76**			**0.91**
	Total	**935**	**77**	**31.05**	**14916**	**140**	**3.65**
C44	500Gy	257	26	10.12	4150	41	0.99
	600Gy	251	26	10.36	4272	45	1.05
	0.3%EMS	412	35	8.50	5506	54	0.98
	0.4% EMS	290	45	15.52	4138	86	2.08
	Overall			**11.13**			**1.28**
	Total	**1210**	**132**	**44.5**	**18066**	**226**	**5.1**
Pb. 1	200Gy	367	21	5.72	5519	22	0.40
	300Gy	345	4	1.16	5133	7	0.14
	0.2%EMS	668	51	7.63	9433	63	0.67
	0.3%EMS	615	53	8.62	8100	71	0.88
	Overall			**5.78**			**0.52**
	Total	**1995**	**129**	**23.13**	**28185**	**163**	**2.08**
CH40/91	200Gy	252	27	10.71	3219	41	1.27
	300Gy	143	34	23.78	1678	69	4.11
	0.2%EMS	92	11	11.96	980	19	1.94
	0.3% EMS	112	13	11.60	1235	23	1.86
	Overall			**14.51**			**2.30**
	Total	**599**	**85**	**58.05**	**7112**	**152**	**9.18**
	G. Total	**4739**	**423**	**156.7**	**68279**	**681**	**20.01**

Table. 2. Total (pooled) frequency of morphological (macro) mutants induced in M_2 generation of chickpea genotypes.

Mutagen basis (mutagens pooled over varieties)		Relative frequency (%) of morphological mutants				
Mutagens	**Total no. of M_2 plants**	**Pb2000**	**C44**	**Pb-1**	**CH40/91**	**Total**
Gamma	31981	1.38	1.02	0.27	2.69	**1.34**
EMS	36298	0.45	1.53	0.78	1.90	**1.17**
Total	**68279**	**1.83**	**2.55**	**1.05**	**4.59**	**2.51**

The trend of increasing frequency of morphological mutations with the increase in the doses/concentrations of both mutagens (gamma and EMS doses) was exhibited only in desi genotype C44, gamma irradiation doses in CH40/91 and EMS doses in Pb-1. Whereas the decreasing trend was exhibited with the increase in doses of both mutagens in Pb2000, gamma irradiation doses in Pb-1 and EMS doses in CH40/91. With the increase in dose of gamma rays, decrease in mutation frequency was observed in soyabean by Kadam (2000). In Pb2000 the 'saturation effect' was attained at 300Gy, in Pb-1 at 200Gy and in CH40/91 at 0.2% EMS treatment (Swaminathan, 1961). The reduction in mutation frequency at 400Gy in Pb2000, at 300Gy in Pb-1 and at 0.3% EMS gamma irradiation treatments in CH40/91 as compared to higher doses may be due to the result of elimination of mutations associated with gross chromosomal changes through haplontic and diplontic sieves (Swaminathan 1961). Viable morphological mutation frequency ranged from 1.16 to 23.78% on M_1 progeny basis and 0.27 to 4.11% on M_2 population basis. Gamma irradiation treatment exhibited overall highest morphological mutation frequency on M_1 progeny basis in three genotypes (Pb2000, C44 and CH40/91) while the EMS treatment exhibited overall highest morphological mutation frequency in two genotypes (C44 and CH40/91). Two genotypes (Pb2000 and Pb-1) exhibited overall least morphological mutation frequency in EMS treatments. On M_2 population basis, gamma irradiation treatment exhibited overall highest morphological mutation frequency in two genotypes (Pb2000 and CH40/91) whereas in EMS treatment overall highest morphological mutation frequency was exhibited in three genotypes (Pb2000, C44 and CH40/91). On overall basis, Pb-1 exhibited lowest frequency of morphological mutants (0.52%) as well as chlorophyll (1.23%) mutants while CH40/91 exhibited highest frequency of morphological mutants (2.30%) (Table 1) as well as chlorophyll (2.13%) mutants (Shah *et al.*, 2006). The significant higher morphological as well as chlorophyll frequency of CH40/91 as compared to other three genotypes could be due to diverse nature of genotype as it was recombinant genotype of desi x kabuli while the other genotypes are pure desi and kabuli type genotypes in origin. The differential spectrum of morphological mutations has been reported in chickpea by Kharkwal (1999). Comparative studies on morphological characteristics revealed that the chemical mutagens, particularly alkylaing agents are more effective than ionizing radiations (Solanki & Sharma, 1999; Kharkwal, 2001). In another case, Kharkwal (1999) reported that chemical mutagens induced higher frequency of mutations than the gamma rays in chickpea varieties. It is possible that chemical mutagens may prove to be a better alternative for inducing morphological mutations, as they induce mutations at a much higher rate and cause less chromosomal disturbances than radiations (Sharma, 2001). The higher mutagenic sensitivity of gamma rays has been reported in soyabean by Wagmare & Mehra, (1998) as compared to EMS.

The proportion of chlorophyll mutations (Shah *et al.*, 2006) was much greater than that of morphological mutations being 916:681. It was observed that the proportion of morphological mutations was lesser than that of chlorophyll mutants being 1:1.25. Whereas Kharkwal *et al.*, (1988) and Haq (1990) have reported that the proportion of morphological to chlorophyll mutations was about 1:2 in their studies on legumes. The contradiction in results may be due to the difference among chickpea genotypes and type of mutagens used.

The correlations between different types of chlorophyll mutants and morphological mutants in M_2 population are presented in Table 3. There was a significant positive correlation between appearance of albina and xantha and highly significant positive correlations between albina and viridis, xantha and chlorina, xantha and viridis and chlorina and viridis. All the chlorophyll mutant types showed highly significant positive correlation with total chlorophyll mutants whereas only albina and other mutants showed significant positive correlation with total morphological mutants. Data have shown non-significant correlation between the appearance of chlorophyll and morphological mutations and thus suggests that the occurrences of both the types are independent of each other. Present results confirm the findings of Karve (2003) who did not find any chlorophyll mutations in any of the four doses of gamma rays in Senna (*Cassia angustifolia*). On the other hand, he selected good number of morphological mutants for various characters.

In addition to large number of single morphological mutations, multiple mutations affecting two or more characters were found common in all the mutagenic treatments in the present study. Their frequency varied with genotypes and mutagens used. Relative frequency of progenies segregating for single and multiple macromutations are presented in Table 4. Majority of the progenies segregated for three types of mutation. The proportion of segregating progenies ranged from 70.13 to 95.46% for one type, 1.40 to 16.88% for two types and 1.16 to 17.77% for three types of morphological mutants. Almost all the progenies segregated for multiple mutations except only one progeny in Pb-1, which segregated for two types of morphological mutations in 200Gy treatment. The gamma irradiation dose of 500 Gy proved to be the most effective (94.46%) to induce type I mutations, 0.3% EMS (16.88%) in type II and 600Gy gamma irradiation dose (17.77%) in type III**.** Kharkwal (1999) observed lowest frequency of multiple mutants in kabuli type variety C104 and very high in desi var. G130 and H214. He also found gamma irradiation more effective in inducing multiple mutations. Occurrence of multiple mutations has also been reported in groundnut by Gregory (1968). Patil (1966) attributed this phenomenon to either mutation of pleiotropic gene, or mutation of gene clusters or due to the loss of the chromosomal segments. Gaul (1960) interpreted the occurrence of such mutants as due to chromosomal rearrangement or deletion.

Table 3. Correlation between different types of chlorophyll (micro) and morphological (macro) mutants in M_2 generations.

		Albina	Xantha	Chlorina	Viridis	Others	Total chlorophyll	Total morphological
1.	Albina	1.000						
2.	Xantha	0.614*	1.000					
3.	Chlorina	0.336	0.736**	1.000				
4.	Viridis	0.637**	0.940**	0.662**	1.000			
5.	Others	0.773**	0.321	0.014	0.271	1.000		
6.	Total chlorophyll	0.676**	0.976**	0.815**	0.950**	0.343	1.000	
7.	Total morphological	0.599*	0.127	-0.055	0.258	0.587*	0.205	1.000

d.f = 14

Table 4. Relative frequencies of M_2 segregating population for varying number of morphological (macro) mutations.

Genotypes	Treatment	Type I	Type II	Type III
Pb. 2000	300Gy	85.71	12.51	1.78
	400Gy	86.96	6.52	6.52
	0.3%EMS	66.66	16.67	16.67
	0.4% EMS	76.92	7.69	15.39
C44	500Gy	92.68	4.88	2.43
	600Gy	77.78	4.45	17.77
	0.3%EMS	92.59	1.85	5.56
	0.4% EMS	89.53	9.31	1.16
Pb 1	200Gy	95.46	4.54	0.00
	300Gy	71.42	14.29	14.29
	0.2%EMS	93.66	3.17	3.17
	0.3%EMS	94.37	1.40	4.23
CH40/91	200Gy	82.93	12.19	4.88
	300Gy	94.20	4.35	1.45
	0.2%EMS	76.55	13.58	9.80
	0.3%EMS	70.13	16.88	12.99

Based upon this findings, three genotypes (Pb2000, C44 and Pb-1) can be considered to be genetically close. The present results also suggest that induced mutability is governed by the genetic architecture of the material used. The genotypic control of the mutation process as observed in the present studies has been reported in different crops by Gumber *et al.,* (1995), Kharkwal (1999) and Khan *et al.*, (2005). The CH40/91 in the course of present study was found to be relatively resistant to mutational changes in general. There were also differences in mutation spectrum between the two mutagens. The more frequent induction of certain mutation types by a particular mutagen may be attributed to the fact that the genes controlling these characters may be more responsive to either alkylating agents or ionizing radiations. This could be due to differential mode of action of the mutagens on different base sequences in various genes. Nilan (1967) concluded that different mutagens and mutagen treatment change the relative proportion of different mutation types. Differences in the frequency of various morphological induced mutations have been reported (Tripathi & Dubey, 1992; Vandana & Dubey, 1994). Although the full possible range of EMS concentrations was not employed, it can still be concluded from the present study that gamma rays and EMS differ in their mutation spectra.

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