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RESEARCH ARTICLE

GENETIC VARIABILITY OF MORPHOLOGICAL MUTANTS INDUCED BY GAMMA RAYS AND EMS IN CHICKPEA (VARIETY-VIJAY AND PKV-2)

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ABSTRACT

Chickpea is an important grain legume cultivated worldwide. Both *desi* and *kabuli* biotypes are widely used as prime source of protein in many countries of the world. A narrow genetic base is one of the major bottlenecks in chickpea improvement programs. Induced mutations can be an effective way to introduce variability in the existing germplasm/cultivars for their effective utilization in the breeding programs. Genetic variability of morphological mutations induced by varying doses of physical (gamma rays) and chemical (EMS) mutagens in M_2 population was studied in one *desi* (Vijay) and one *kabuli* (PKV-2) varieties of chickpea. In M_2 population, 42 different types of morphological mutations in different parts of the plants, such as growth habit, branching pattern, stem structure, foliage type architecture and color, plant height, pod and seed size, flower color, flowering behavior and maturity was observed. Further the mutants were also grouped on the basis of variability observed in single, two or multiple traits. EMS was found to be more effective than gamma rays in induction of chlorophyll variations in both the cultivars. Overall lower doses of both mutagens were found to induce more variation as compare to higher doses. The mutations per 1000 M_2 progeny was recorded highest in 300 Gy gamma radiations and 0.2% EMS for variety Vijay and 150 Gy gamma radiations and 0.2% EMS in variety PKV-2. Both gamma rays and EMS were found to have significant mutagenic potential to induce morphological variations in Chickpea.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop widely cultivated and consumed at global level. It is a member of Fabaceae with diploid chromosome number 2n=2x=16 and is highly self-pollinated with an outcrossing rate of less than 1% (Arumuganathan and Earle, 1991). It is an essential and cheap source of protein, carbohydrate and minerals in human diet especially in Indian subcontinent (Jukanti *et al.,* 2012) and plays a key role in the enrichment of soil fertility by fixing atmospheric nitrogen through symbiotic nitrogen fixation. The average yield of chickpea reported in India so far is far below than its potential (Choudhary *et al*., 2013). Worldwide efforts are being made to improve the qualitative and quantitative traits of this crop. However, its narrowing genetic base is reportedly the major cause of concern for the breeding programs for chickpea improvement as well as for the crop production and productivity in the climate change scenario as a narrow genetic base also increase the vulnerability of this crop to various biotic and abiotic stresses (Sharma *et al*., 2013, Joshi-Saha and Reddy, 2014). Induced mutation is a vital tool used for the improvement of crops through the introduction of mutations at different loci that regulates economically important traits and/or by removing undesirable genes from elite breeding lines (Lippert *et al.,* 1964). Use of mutation breeding to create genetic variability in existing gene pool or to develop characters which are unavailable or lost from the existing gene pool is a very promising breeding activity. Mutation breeding has additional advantage when there is a case of improvement of a good variety which needs to alter just one or two traits (Joshua, 2000). Genetic variability for desirable traits can be effectively induced through mutations and its practical significance in plant improvement programmes has been well recognized (Gaur and Gour, 1999; Atta *et al.,* 2003; Nayyar *et al.,* 2005; Ganapathy *et al*., 2008; Joshi Saha *et al.,* 2015). Induced morphological mutants are used as markers in genetics, physical and biochemical investigations of gene action of mutagenic factors (Gaul, 1964). The frequency and spectrum of chlorophyll mutants are being used as the primary index to test effectiveness of mutagens and mutability of genotype which in turn would be useful to generate the wide array of useful mutants in treated population. Mutagens have been used to induce useful phenotypic variations in crop plants.

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Morphological mutations affecting different plant parts can be of enormous practical utility to isolate and further develop some novel mutant lines and many of them have been released directly as crop varieties (Shah *et al*., 2010; Joshi Saha *et al*., 2015). 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 study was undertaken to observe the phenotypic as well as genotypic alterations induced through the application of physical and chemical mutagens in two chickpea biotypes (*desi* and *kabuli*) represented by two popular varieties of chickpea.

MATERIALS AND METHODS

Germplasm of chickpea *desi* variety Vijay (Phule G-81-1-1) and *kabuli* variety Kabuli-2 (PKV-2) was procured from Mahatma Phule Agricultural University Rahuri and Dr. Panjabrao Deshmukh Agriculture University, Akola (MS) respectively. The dried, healthy seeds with 10-12% moisture content were irradiated with gamma rays with dose of 300, 400 and 500 Gy (for Vijay) and 150, 200 and 300 Gy (for PKV-2). For each dose about 150 g seeds of each variety were taken. The gamma ray (GR) irradiation facility (Co^{60} source) was made available from Bhabha Atomic Research Center, Trombay, Mumbai. For EMS treatment about 200 seeds of each variety were presoaked in distilled water and then subjected to different concentrations of ethyl methane sulphonate ranging from 0.2% to 0.4%. The treated seeds were sown in October 2015 under field conditions at Departmental field of Shri Shivaji College, Akola (MS) with spacing 15 cm within row and 30 cm between rows to raise M_1 plants. The M_1 plants were harvested individually and sown in October 2016 to raise M_2 plant to row progenies. The untreated control was sown on either side of each plot. The M_2 population was screened for chlorophyll and other morphological mutations 10 days after germination (Khan *et al.,* 2005). The chlorophyll mutants were identified as per Gutafsson (1940). In addition, other morphological mutants were observed throughout the crop span were tagged and harvested individually. The frequency of mutation was calculated as described by Kharkwal (1998).

RESULTS AND DISCUSSION

From M_2 generation of chickpea variety Vijay and PKV-2, 42 different types of morphological mutants were isolated. These includes 5 different types of chlorophyll mutants (albina, xantha, chlorina, viridis and tigrina) (fig. 1, A: a-e), with highest frequency of tigrina mutants. The recorded highest frequency of chlorophyll mutant was in 400 Gy Gamma rays and 0.3% EMS in Vijay and 200 Gy Gamma rays and 0.4% EMS in PKV-2 (in press). Apart from chlorophyll mutants, other morphological mutants like erect plant type, dwarf mutants, gigas, spreading, prostrate, mutants with leaf variations, flower color and early flowering (Fig.1 B: f-q). The frequency of above mutants isolated from M_2 population is given in Table 1 and 2. The types of mutations and the trends observed in terms mutation frequencies were found to be genotype specific. The erect plant types were isolated only from *desi* variety Vijay, with highest frequency in gamma irradiation (300 Gray) derived population. This dose also showed highest frequency of dwarf mutants. In case of EMS, 0.4% concentration was found to induce highest dwarfism. Throughout the entire population of Vijay, only four gigas were observed; two in 300 Gy, one in 400 Gy and one in 0.3% EMS (Fig-1. B: f and g; Table-1). No gigas was identified in PKV-2. Leaf variation is one of the most common phenotypic changes that could be induced by mutagens. In variety Vijay the highest frequency of induced leaf variation (Fig.1. B: h –k) was shown by EMS (0.2%). In gamma induced population the mutation frequency for leaf mutations was highest in 500 Gy i.e. the highest dose used in the present study, while in EMS induced population, EMS at0.2% concentration induced highest leaf variations as compared to the higher doses of 0.3% and 0.4%(Table-1). In PKV-2, 0.3% EMS was found to induce highest frequency of leaf variation followed by 200 Gray gamma radiations.

In case of spreading and prostrate mutants (Fig.1. B: o) 300 and 400 Gy gamma rays respectively produced higher mutation frequency in *desi* cultivar Vijay while, mutation frequencies of such growth habit mutants were less in *kabuli* variety PKV-2 and were induced at lower doses of gamma rays (i.e. 150 and 200 Gy respectively) (Table 1,2). Similar trend was observed in case of EMS mutagenesis, where lower EMS doses of 0.2% and 0.3% induced more growth habit mutants in both *desi* and *kabuli* cultivars (Table 1,2). Flowering is an important agronomic character of crops, as 'days to flowering' decides the duration of crop cultivation. Both gamma radiations and EMS doses selected for present study were found to induce earliness in flowering. 54 mutant plants flowered 10-20 days earlier than respective control. Mutation frequencies of early flowering mutants showed a dose dependent response in both *desi* and *kabuli* cultivars with more frequency of mutants observed in higher doses of 500 Gy and 300 Gy in Vijay and PKV-2 respectively (Table 1,2). However, no such trend was observed in case of EMS mutagenesis with 0.4% and 0.2% EMS showed highest mutation frequencies for early flowering in Vijay and PKV-2 respectively (Table 1, 2). Flower color variations were recorded only in 300 Gy and 400 Gy gamma rays and 0.3% EMS induced populations of variety Vijay. In case of PKV-2, only 150Gray was found to induce flower color variations (fig.1. B: p –q), however, the frequency was very low (table-2), where the plant was dwarf with pink colored flowers.

Mutants screened for variability in different characters were then grouped into three types based on whether the change was observed in one trait (Type I), two traits (Type II) or multiple traits (Type III). It was observed that, the M_2 progeny of both Vijay and PKV-2 showed higher frequency of Type I mutants (Table-3). Overall, total morphological mutation frequencies per 1000 M_2 progeny was highest for 0.2% EMS in both *desi* and *kabuli* cultivars, while for gamma rays, lowest dose of 300 Gy and lowest dose of 150 Gy produced maximum morphological mutation frequencies in *desi* cultivar Vijay and *kabuli* cultivar PKV-2 respectively. The spectrum and frequency of induced morphological mutants are both mutagen and biotype dependent in chickpea. EMS was previously found to induce a higher frequency of chlorophyll mutations in both the varieties (Bogawar *et al.,* 2017) however, variety Vijay was observed to be more sensitive than PKV-2. Both varieties also differ drastically in their phenotypic appearance; former is small, semi-spreading *desi* variety with small seeds while later have tall semi-spreading *kabuli* type with bold seeds. The differences in genetic makeup have its own independent impact on the rate of mutation and rate of recoverable spectrum of mutants (Ahsan- ul-Haq *et al.,* 1994; Kaul & Bhan, 1997; Khan *et al,* 2005 and Joshi Saha *et al,* 2015).

Fig:1. Photographs of some morphological mutants isolated from M_2 Population of Chickpea

A: Chlorophyll mutants

B: Other morphological mutants

A: Chlorophyll mutants:-of Vijay and PKV-2 (a-e)
B: Other morphological mutants:- f: Gigas : Vijay (0.2% EMS), g: Gigas : Vijay (300 Gray GR), h: Leaf variant : Dimorphic Vijay (300 Gray GR), i: Leaf variant: Vijay (400 Gray GR), j: Leaf & Habit variant : Vijay (300 Gray GR), k: Leaf variant, dwarf : Vijay (300 Gray GR), l: Early flowering, maturing: Vijay (400 GR), m: Prostrate mutant: PKV-2 (200Gray GR), n: Dwarf, sterile mutant: Vijay (500Gray GR), o: Spreading mutant: Vijay (300 Gray GR), p: Flower color mutant : Vijay (300 Gray GR), q: Multiple trait mutant: PKV-2 (150 Gray GR)

Table 1. Frequency of different morphological mutants isolated from M_2 progeny of Chickpea variety- Vijay

Dose	No. of M_1 Plants	No. of M_2 Plants	Frequency of other morphological mutants								Mutations per 1000 M_2 progeny
			Er	Dw	Gig	L V	Spr	Pros	EF	FlC	
GR300Gy	281	7380	0.298	1.00	0.040	0.365	1.409	0.054	11.25	0.162	284.95
GR400Gy	260	4080	0.294	0.318	0.024	0.294	0.735	0.073	4.656	0.098	97.05
GR500Gy	200	1502	00	0.998	00	0.466	0.532	0.066	1.72	00	77.53
EMS 0.2%	245	1480	0.135	0.202	0.135	0.810	1.013	0.135	4.864	00	138.51
EMS 0.3%	220	1460	0.273	0.410	00	0.547	0.173	0.136	3.698	0.205	89.04
EMS 0.4%	185	1152	00	0.347	00	0.260	0.410	0.086	9.114	00	108.50

Note: Er: Erect mutants, Dw: Dwarf mutants, Gig: Gigas, LV: Leaf variation in architecture, Spr: Spreading mutant, Pros: Prostrate mutant, EF: Early flowering mutants, FLC: Flower colour mutants.

Table 2. Frequency of different morphological mutants isolated from M_2 progeny of Chickpea variety- PKV-2

Dose	No. of M_1 Plants	No. of M_2 Plants	frequency of other morphological mutants								Mutations per 1000 M_2 progeny
			Er	Dw	Gig	L V	Spr	Pros	EF	FlC	
GR150Gy	112	945	00	0.507	00	0.211	0.423	00	5.71	0.211	172.48
GR200Gy	108	630	00	*0.317*	00	0.37	0.158	0.317	3.49	00	47.61
GR300Gy	130	571	00	0.875	00	00	00	00	8.05	00	87.56
EMS 0.2%	116	608	00	26.48	00	0.164	0.328	0.164	14.80	00	151.31
EMS 0.3%	098	518	00	0.579	00	0.386	0.579	00	2.89	00	34.74
EMS 0.4%	072	413	00	0.484	00	0.242	00	00	2.66	00	29.05

Note: Er: Erect mutants, Dw: Dwarf mutants, Gig: Gigas, LV: Leaf variation in architecture, Spr: Spreading mutant, Pros: Prostrate mutant, EF: Early flowering mutants, FLC: Flower colour mutants.

Table 3. Relative frequencies of M_2 segregating population for varying number of morphological mutants

Variety	Treatment/ doses	Type-I	Type-II	Type-III
Vijay	GR300Gy	71.26	18.50	10.24
	GR400Gy	82.52	12.18	05.30
	GR500Gy	85.21	09.69	05.10
	EMS 0.2%	62.78	21.66	15.66
	EMS 0.3%	69.25	18.29	12.46
	EMS 0.4%	73.25	23.66	03.09
PKV-2	GR150Gy	86.21	12.06	01.63
	GR200Gy	88.52	11.02	0.46
	GR300Gy	89.15	08.75	02.10
	EMS 0.2%	72.68	18.22	09.10
	EMS 0.3%	76.55	22.18	01.27
	EMS 0.4%	78.13	19.36	02.51

Though, EMS was observed to be most effective mutagen for induction of chlorophyll mutations (Bogawar *et al.,* 2017), present study indicates superiority of gamma rays in induction of morphological mutants for both the cultivars in terms of overall mutation frequency per 1000 M_2 progeny (Vijay: 459.53 (GR) and 336.05 (EMS); PKV-2: 307.65 (GR) and 215.1 (EMS). The comparative superiority of chemical mutagen over gamma rays producing a higher frequency and spectrum of chlorophyll mutations suggest that the chemical mutagens are more efficient in inducing mutations related to chlorophyll development. Swaminathan *et al.* (1962) proposed that such high frequency is due to the preferential action of EMS on chlorophyll development genes located near centromere. Some previous reports showed higher mutagenic efficiency in EMS dose followed by gamma rays (Kharkwal, 1998; Wani 2009). Earlier reports (Shah et al, 2011 and Barshile and Boddu, 2012) on other morphological mutants spreading type, gigas, early flowering were also in analogy with the present study. However, on the basis of frequency patterns of morphological mutants obtained in present study, it seems that the strong mutagens reach their saturation point event at lower doses in highly mutable genotypes and its increase in dose does not add to the mutation frequency. With increase in dose beyond a critical point, the strong mutagens become more toxic that the higher doses of relatively weaker mutagens. The type grouping of all morphological mutants indicates that both (Vijay and PKV-2) are genetically different and might not share common loci for same characters (Shah *et al.,* 2011). From the current study, it is clear that both gamma rays and EMS induce potential variability in Chickpea varieties that could be assessed visually in the form of morphological mutations. The mutants identified in the present study will be useful in further breeding programs and they also constitute an important collection for basic studies and gnetic characterization of loci regulating these agromorphological traits.

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