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RESEARCH ARTICLE

Frequency and spectrum of chlorophyll mutants induced by gamma rays and EMS in two chickpea varieties (Variety- Vijay and PKV-2)

■ **PRAJWAL BOGAWAR, DEEPAK KOCHE AND ARCHANA JOSHI-SAHA**

SUMMARY

Chickpea is one of the most important leguminous food grain grown worldwide. Mutagenesis could be used as a classical way to increase genetic variability in chickpea considering its narrowing genetic base. Present study was an attempt to analyze the frequency and spectrum of chlorophyll mutations induced by gamma rays (300, 400 and 500 Grey) and ethyl methanesulphonate (0.2%, 0.3% and 0.4%) in M_2 generation of varieties of chickpea (Var- Vijay and PKV-2). Broad spectrum chlorophyll mutants were isolated from M_2 generation. The relative frequency of these mutants in both varieties was observed in order of Tigrina > Viridis > Chlorina > Xantha and Albina. The total chlorophyll mutation frequency increased with increase of dose upto certain limit and then started declining. However, the effectiveness of EMS was found to be more prominent than gamma rays especially in inducing chlorophyll mutations in Chickpea.

Key Words : Chickpea, Chlorophyll mutants, Ethyl methanesulphonate, Gamma rays, Frequency

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Chickpea (*Cicer arientinum* L.) is a good source of protein, carbohydrates, minerals and fibres (Jukanti *et al*., 2012) and is being consumed for its nutrition worldwide, thus, having status of second largest leguminous crop cultivated in 13.2 Million hectare area with production of about 11.6 Million tones (FAO STAT, 2013). The average yield of chickpea reported so far is far below than its potential and conventional breeding does not offer any solution to increase its productivity (Choudhary *et al*., 2013). Worldwide efforts are being made to improve the qualitative and quantitative traits of this crop. But its narrowing genetic base is reportedly the major cause of concern leading vulnerability of this crop to biotic and abiotic stresses (Sharma *et al*., 2013 and Joshi-Saha and Reddy, 2014).

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However, mutation breeding could be a possible way to increase the genetic variability of this crop (Barshile and Boddu, 2012).

Induced chlorophyll mutants are considered 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. Since last seven decades more than 2,252 mutant varieties including that of cereals, pulses, oilseeds, vegetables and other economically useful plants were released all over the world (Maluszynski *et al.*, 2000). In case of chickpea, few workers have started working on its improvement though mutation breeding (Haq *et al.*, 2002, Kharkwal, 2003 and Toker and Cagigran, 2004) in the beginning of 21st century.

The chlorophyll mutations are not economic but could be use to identify threshold dose of mutagen that would increase genetic variability (Joshua, 2000). These are the most frequently observed factorial mutations in M_2 generation. Present study was focused on screening of chlorophyll mutants from M_2 population of chickpea (var- Vijay and PKV-2) derived from mutagenized M_1 plants.

MATERIAL AND METHODS

Germplasm of chickpea, variety Vijay (Phule G-81-1-1) and variety Kabuli-2 (PKV-2) was procured from Mahatma Phule Agricultural University Rahuri and Dr. Panjabrao Deshmukh Agriculture University, Akola (M.S.), respectively. The dried, healthy seeds with 10-12 per cent moisture content were irradiated with gamma rays with dose of 300, 400 and 500 Gray (for Vijay) and 150, 200 and 250 Gray (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 per cent to 0.4 per cent.

The treated seeds were sown in October 2015 under field conditions at Departmental field of Shri Shivaji College, Akola (M.S.) 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 mutations 10 days after germination (Khan *et al*., 2005). The chlorophyll mutants were identified as per Gustafsson (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). Effectiveness and efficiency of different mutagens were calculated as per Konzak *et al.* (1965).

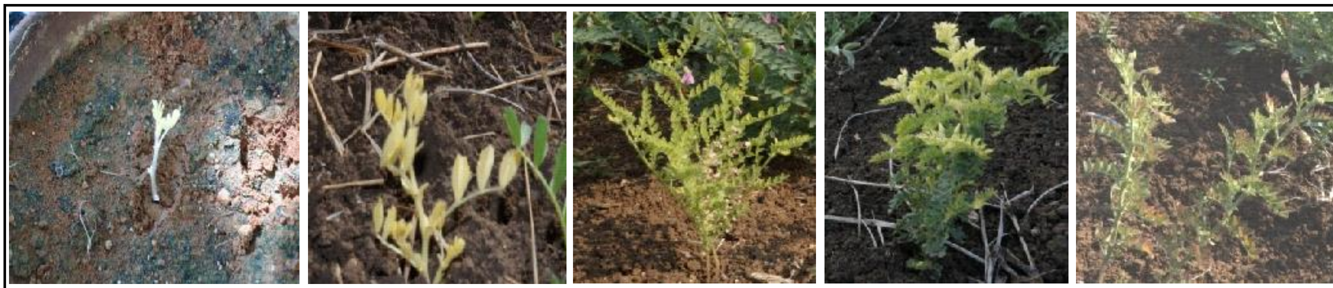
RESULTS AND DISCUSSION

Being useful markers to assess the potential of mutagens, chlorophyll mutants were scored from M_2 population. No spontaneous chlorophyll mutants were observed in untreated control populations. Five different types of chlorophyll mutants were isolated from 10- 20 day old M_2 population. The spectrum of different M_2 chlorophyll mutants included; albino, chlorina, xantha, viridis and tigrina (Fig. 1, A, B, C, D and E). Albina and xantha mutants were dead after 03 to 04 weeks, rest survived but tigrina and viridis were with less number of branches; however, chlorina mutants were survived like normal plants.

The data on mutation frequencies and spectrum of induced chlorophyll mutants in cultivars Vijay and PKV-2 is presented in Table 1 and 2. Overall spectrum of chlorophyll mutants isolated showed the highest frequency of tigrina mutants followed by viridis and least was of albina. We did not observed any albina mutant in gamma ray irradiated population of Vijay, but treatment of 0.2 per cent and 0.4 per cent EMS showed albina traits (Table 1). In case of gamma irradiated M_2 population 400 Gray was observed to induced highest mutation frequency of chlorophyll mutants and then start declining. In EMS induced population, 0.3 per cent dose was found to be most effective (Table 1). While in PKV-2 variety, 250 Grey dose of gamma rays and 0.4 per cent dose of EMS was most effective (Table 2). However, comparing the total relative frequencies for chlorophyll mutants, EMS was found to be more effective than gamma rays (Table 3).

The chlorophyll spectrum and frequency induced are both mutagen and variety dependent in chickpea. In present study EMS was found to induce the higher frequency of chlotophyll mutations in both the varieties however, variety Vijay was observed to be more sensitive than PKV-2. This might be due to genotypic differences in selected varieties. Variety Vijay and PKV-2, 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 and Bhan, 1977; Khan *et al.*, 2005 and Joshi Saha *et al.*, 2015). The maximum induction of tigrina mutants followed by viridis indicates that the genes for chlorophyll development might have mutations which does not synthesize sufficient amount of chlorophylls and thus negatively affecting overall development of plant.

Though, EMS was observed to be most effective mutagen, gamma rays also induced significantly higher number of scoreable chlorophyll mutants. The comparative superiority of chemical mutagen over gamma rays producing a higher frequency and spectrum of



A Albina : PKV-2 (150 Grey) B Xantha :PKV-2 (200 Grey) C Clorina : Vijay (0.3 % EMS) D Viridis : Vijay (300 Grey) E Tigrina :Vijay (400 Grey)

Fig. 1 : Photographs of some chlorophyll mutants isolated from M_2 population of chickpea

Table 1 : Relative frequencies of chlorophyll mutations induced by gamma rays and EMS in chickpea (Variety- Vijay)

Treatments /Dose	No. of M2 plants	Relative frequency (%) of chlorophyll mutants					Total frequency (%)	Mutagenic effectiveness
		Albina	Chlorina	Xantha	Viridis	Tigrina		
G300	7380	00	0.094	0.040	0.243	1.978	2.355	0.078
G400	4080	00	0.073	0.024	0.294	3.235	3.626	0.090
G500	1502	00	0.199	0.066	0.599	2.050	2.914	0.058
E0.2	1480	0.071	0.135	0.202	0.810	2.121	3.339	1.391
E0.3	1460	00	0.068	0.136	0.753	2.876	3.833	1.064
E0.4	1152	0.173	0.086	0.173	1.128	1.909	3.469	0.722

Table 2 : Relative frequencies of chlorophyll mutations induced by gamma rays and EMS in chickpea (Variety- PKV-2)

Treatments / Dose	No. of M2 plants	Relative frequency (%) of chlorophyll mutants					Total frequency (%)	Mutagenic effectiveness
		Albina	Chlorina	Xantha	Viridis	Tigrina		
G150	945	0.105	0.211	0.317	0.105	0.211	0.949	0.063
G200	630	00	0.317	0.317	0.158	0.793	1.585	0.079
G250	571	00	0.175	0.350	0.350	0.525	1.590	0.063
E0.2	608	00	0.164	0.328	0.328	0.493	1.313	0.547
E0.3	518	00	0.386	0.590	0.590	0.965	1.941	0.539
E0.4	413	00	0.242	0.242	0.484	0.726	1.691	0.352

Table 3 : Comparison of gamma rays and EMS induced frequencies of chlorophyll mutants in two chickpea varieties

Mutagen	Comparative frequency of chlorophyll mutations (%)					Total frequency (%)
	Albina	Chlorina	Xantha	Viridis	Tigrina	
Gamma rays	0.105	1.069	1.114	1.749	8.792	12.829
EMS	0.244	1.081	1.671	4.093	9.090	16.179
Total average frequency (%)	0.174	1.075	1.392	2.921	8.941	14.504

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 and Wani, 2009). Further, 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.

For plant breeders, mutagenic efficiency is more relevant than mutagenic effectiveness as mutagenic effectiveness is measured considering the biological damage. Similar conclusions were drawn earlier by Konzak *et al*. (1965); Kharkwal (2003); Khan *et al.* (2005); Joshi-Saha *et al.* (2015) and Loyavar *et al.* (2017). 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 chlorophyll mutants.

Conclusion:

Marked differences were observed in the expression of induced chlorophyll mutations at different doses of mutagens due to genetic differences existing between the selected chickpea varieties. Among the mutagens, EMS was more effective than gamma radiations. Overall tigrina type chlorophyll mutations were most frequent and albino were least common. Further, it could be suggested that as both gamma radiations and EMS doses induced significant chlorophyll mutations, both mutagen could be used in mutation breeding for inducing useful variable mutations.

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