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Research Article – Cytogenetics

Induced mutagenesis in Chickpea (*Cicer arietinum* (L.) with special reference to the frequency and spectrum of chlorophyll mutations

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Abstract

A relative study of frequency and spectrum of chlorophyll mutations induced by mutagens in M_2 generation was made with chickpea (Cicer arietinum (L). Variety 'CO-4'. The treatments include different doses/concentrations of Gamma rays (20, 30, 40, 50 and 60kR) and Ethyl Methane Sulphonate (10, 20, 30, 40 and 50 mM). From the study, the overall frequencies and spectrum of five types of induced chlorophyll mutants Viridis (0.55), Xantha (0.46), Chlorina (0.45), Albina (0.43) and Tigrina (0.35) were observed. The frequency of chlorophyll mutation was increased with increasing concentrations up to a level, beyond it declined in both the mutagens. And the chlorophyll frequency was found in the order of viridis > xantha >chlorina> Albina >tigrina. The chemical mutagen, EMS was found to be more effective in inducing chlorophyll mutations than gamma rays in Chick pea.

Key words: Chick pea, Chlorophyll mutation, Albina, Xantha, Chlorina, Viridis, Tigrina, Frequency and spectrum

Introduction

Gaul (1964) clarified that chlorophyll mutants are employed as markers in genetics, physical, and biochemical investigations of gene action of mutagenic factors in inducing mutation studies. The spectrum and frequency in chlorophyll mutants are being used as the primary index of the effectiveness of mutagens and mutability of genotypes towards the mutagen which in turn would be useful to generate the wild array of desirable mutants in the treated population.

The chlorophyll mutations do not have any economic value due to their lethal nature; such a study could be useful in identifying the threshold dose of a mutagen that would increase the genetic variability. Chlorophyll mutants are used as tests for evaluation of genetic action of mutagenic factors (Svetleva, 2003). They are the most frequently observed and can be easily identified factorial mutations in M_2 generation yet. The selection of effective and efficient mutagen(s) is very essential to recover a high frequency and spectrum of desirable mutations.

Improvement in the frequency and spectrum of mutations in a predictable manner and thereby achieving the desired plant characteristic for their direct or indirect exploitation in the breeding programme is an important goal of mutation research. Mutation induction has proven to be a workable, sustainable, highly efficient, environmentally acceptable, flexible, unregulated, non – hazardous and a low cost technology in the breeder's tool box to enhance crop improvement.

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Materials and Methods

Mutagenic treatment: Seeds of Chick pea, 'CO-4' variety from Tamilnadu Agricultural University, Coimbatore was used for the present study. For EMS treatment, the seeds were pre- soaked in distilled water for 6 hrs, were subjected to different concentrations of ethyl methane sulphonate ranged from 10 to 50mM. For gamma rays treatment, the seeds were irradiated with different doses (20 – 60kR) from ^{60}Co gamma cell in Indira Gandhi Atomic research Centre, Kalpakkam.

Raising M_1 generation: For raising M_1 generation, the seeds were treated with different doses/concentrations of gamma rays and EMS and were sown along with controls at the Botanical Garden, Department of Botany, Annamalai University in a complete Randomized Bloch Design (CRBD). The seeds were harvested separately and randomly from healthy individual of M_1 plants.

The M_2 generation was grown from single plant M_1 progeny seeds. The frequency and spectrum of

the different chlorophyll mutants were scored in M_2. They were identified and classified according to Gutafsson (1940).

Results

Six different types of chlorophyll mutants were isolated in the field in M_2 generation when seedlings were 10 – 20 days old. The spectrum of different M_2 chlorophyll mutants included; albino, chlorina, tigrina, viridis and xantha. A brief description of the isolated chlorophyll mutants was given in Table-1 (Fig.1).Albina and xantha mutants were dying within 10 to 20 days after emergence. In few cases chlorina mutants were survived, but they were vigorously growing. The tigrina and viridis mutants survived and were vigorously growing had few branches with weak stem and low number of pods per plants.

From the relative frequency and spectrum of chlorophyll mutations produced in each treatment (Table-2.). It is evident that both gamma rays and EMS induced a wide spectrum of chlorophyll mutations. The tigrina mutant was least in number in gamma irradiation as well as EMS treatment. Out of 191 chlorophyll mutants only 30 mutants (16 from gamma irradiation and 14 from EMS) were tigrina. Compared to chlorina, Xantha and viridis, the frequency of albina mutants were less. From the results, it was observed that, out of 191 chlorophyll mutations only 37 mutants (17 from gamma irradiation and 20 from EMS) were albina. The overall frequency of viridis (0.55) mutants was relatively high in both gamma irradiation and EMS followed by xantha (0.46), chlorina (0.45), albino (0.43) and tigrina (0.35). Thus the trend of chlorophyll frequency was in the order viridis > xantha >chlorina> albina >tigrina.

A linear relationship between mutation frequency and the dose of gamma rays and EMS was observed up to 40kR in gamma rays and 30mM of EMS treatments.

Fig.1. Showing chlorophyll mutants in the treated population of Chick pea in M2 generation

Albina (50Mm)

Chlorina (30kR)

Xantha (20KR)

Tigrina (40kR)

Viridis (30mm)

The frequency of albina and tigrina mutants were observed higher in gamma irradiation than the EMS treatments.

Table1: Characteristics failure of Chlorophyll mutants and percentage mutated plant progenies induced by gamma rays and EMS in 'CO – 4' variety of Chick pea in M_2 generation.

Isolate mutant types and their characteristics	Treatment	Number of M_1 plat progenies	Number of plant progenies segregating in M_2	% of Mutated plant progenies(M_p)
1. Albina	Control	50	-	-
Lethal mutant, characterized by entirely white leaves of seedlings and the seedlings survived for 10 to 12 days after germination.	20kR	50	2	4.00
	30kR	50	4	8.00
2. Chlorina	40kR	50	5	10.00
Light green colour of leaves; most of the seedlings died within 20 days.	50kR	50	7	14.00
3. Xantha	60kR	50	8	16.00
Leaves were bright yellow in colour; seedling survived for 10 – 20 days.	10mM	50	1	2.00
4. igrina	20mM	50	4	8.00
Leaves are yellow with green patches.				
5. Viridis	30mM	50	8	16.00
Reduced plant height and viviridine green colour of leaves; leaflet size reduced; plants were slow growing and had a low seed yield.	40mM	50	7	14.00
	50mM	50	8	16.00

Table 2: Frequency and Spectrum chlorophyll mutants induced by gamma rays and EMS in 'CO – 4' Variety of Chick pea in M_2 generation

Treatments	M_2 Progenies	Albina	Chlorina	Tigrina	Viridis	Xantha	Mutated seedlings	Frequency
Control	894	-	-	-	-	-	-	-
20kR	843	2	-	5	6	3	16	1.89
30kR	864	4	2	3	5	4	18	2.08
40kR	885	2	5	6	6	5	24	2.71
50kR	797	5	3	-	4	3	15	1.88
60kR	787	4	5	2	3	-	14	1.77
10mM	851	5	1	4	6	4	20	2.35
20mM	868	3	5	2	5	7	22	2.53
30mM	900	2	7	6	8	5	28	3.11
40mM	825	5	4	2	4	2	17	2.06
50mM	795	5	6	-	-	4	15	1.88

Table 3: Mutagen induced chlorophyll mutations frequency (%) and Spectrum in 'CO – 4' variety of Chick pea.

Mutagen	Comparative frequency of Chlorophyll mutation Spectrum					Total frequency
	Albina	Chlorina	Tigrina	Viridis	Xantha	
Gamma rays	0.40	0.35	0.38	0.57	0.35	2.08
EMS	0.47	0.54	0.33	0.54	0.56	2.40
Total frequency	0.43	0.45	0.35	0.55	0.46	2.26

However, comparing the overall frequencies the EMS treatments were found to be more effective that the gamma rays treatment. (Table-3)

Discussions

The chlorophyll mutation frequency is useful in assessing the potency of a mutagen, genetic effects of mutagens and estimation of emotional events mainly because of their easy identification. From the breeder's point of view, the frequency of chlorophyll mutants expressed as per cent of M_2 populations seems to be more realistic and helpful. In the present study, a critical comparison of the chlorophyll mutations indicates that the mutation rate, in general increased with an increasing in dose/concentrations of mutagens up to a certain dose/concentration level beyond which is decreased.

This trend was observed in both mutagenic treatments. It seems that the stronger mutagens reach their saturation point even at lower doses/concentrations in the highly mutable genotypes and further increase in dose/concentrations does not add to the mutation frequency. With an increase in dose/concentrations beyond a point, the strong mutagens become more toxic than the higher doses of relatively weaker mutagens.Higher frequencies of chlorophyll mutations with medium or lower doses of mutagens were reported by Srivastava *et al.,* (1973) and Pawar *et al.,* (2010) in different crops including chick pea.

Out of the six types of chlorophyll mutants recorded in M_2 generation, viridis were predominant, followed by xantha, chlorine, Albina and tigrina. Similar patterns of chlorophyll mutations as observed in the present study (viridis > xantha) has been observed by Tariq *et al.,* (2006), Toker & Cagirgan (2004) in Chick pea and solanki & Sharma (2001) in lentil. Ambarkar (1997) reported a predominance of viridis among chlorophyll mutant types in chickpea. The viridis types were predominat than albina, xantha and chlorina types irrespective of the cultivar in rice bean as reported by Prakash & Shambulingappa, (1999). The reason for the appearance of greater number of viridis after xantha may be attributed to involvement of ploygenes in the chlorophyll formations (Ahmad, 1996). Chlorophyll development seems to be controlled by many genes located on several chromosomes (Gaul, 1967) that could be adjacent to centromere and proximal segments of the chromosomes (Swaminathan *et al.,* 1964 : 1965). According to Von Wettstein *et al.,* (1971) nuclear genes control the biogenesis of plastids. The author found that chlorophyll synthesis is under the control of nuclear genes by the products of regulatory genes. Van Harten, (1998) asserts that the chlorophyll synthesis is under the control of nuclear and out nuclear genes. Mutations in these chlorophyll genes may ultimately cause chlorophyll mutations. Swaminathan *et al.,* (1962) and Ramulu (1970) suggested that differences in the mutation spectrum and rate in different genotypes may be due to differences in the location of genes in relation to the controller. These varietal differences in the frequency of chlorophyll mutations indicate the number of genes controlling chlorophyll development may differ in different varieties of chickpea. Such conclusions get support from earlier work that at least 250 – 300 loci for chlorophyll synthesis exist in barley (Ramulu, 1970). Gustafsson, (1963) reported 125 – 150 loci for another type of chlorophyll mutations. The induction of xantha mutation suggests that genes for xanthophylls are readily available for mutagenic action (Tariq et. al., 2006) and the occurrence of chlorina mutants have been attributed to different causes such as impaired chlorophyll biosynthesis, further degradation of chlorophyll and bleaching due to deficiency of carotenoids (Bevines *et al.,* 1992).

In the present study, the frequency of albina and tigrina mutations was much higher in gamma rays than EMS treatment. Athwal *et al.,* (1970) reported that albina constituted the largest single category of mutants observed in gamma ray treated population of one desi and one kabuli chick pea variety. Gutafsson (1963) believed that ionizing radiations produce high frequency of albina mutations while the chemical mutagens produce other types of chlorophyll mutation of cereal crops.

While comparing the overall performance of mutagen indicated that the frequency of chlorophyll mutation was higher in the EMS treatment than the gamma irradiation. The comparative superiority of chemical mutagens over gamma rays producing a higher frequency and spectrum of chlorophyll mutations suggests the chemical mutagens are more efficient in inducing mutations of genes needed for 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. Higher frequency and a wider spectrum of chlorophyll mutants in chemical mutagen have been reported by Bhattacharya, 2003 and Koli and Ramakrishna, 2002.

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