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EFFECT OF MUTAGEN ON FREQUENCY AND SPECTRUM OF CHLOROPHYLL MUTATION IN CHICKPEA (*CICER ARIETINUM* L.) THROUGH INDUCED MUTATION

Manisha G. Nagargoje[1] and Navnath G. Kashid[2]
[1]Research student, Dept of Botany, Vasant Mahavidyalaya Kaij. Dist: Beed (MS) 431123.
[2]Associate Professor & Research guide, Dept of Botany, Vasant Mahavidyalaya Kaij. Dist: Beed (MS)-431123.

***Corresponding Author Email:** manisha.mukta21@gmail.com

ABSTRACT

Mutation breeding can constitute a valuable tool to the conventional breeding methods in widening the genetic base of cultivated germplasm in crops through creation of some useful mutants, henceforth, mutation breeding finds a prominent place in the augmentation and recreation of genetic variability and has played a significant role in the development of many crop varieties. The frequency of chlorophyll mutation is useful in assessing the potency of mutagen and it is also an indicator of factor mutation. It is considered as a dependable index for evaluating genetic effects of mutagenic treatments. Chlorophyll mutation helps in the study of the effects of specific genes products in differentiations. Mutations in genes responsible for chlorophyll synthesis bring outs deficiency of chlorophyll pigments. The mutagenic effectiveness is reflected in the segregation of chlorophyll mutants and it is also a good indicator to forecast the spectrum of genetic variability that can arise from the mutated sectors. In present investigation a wide spectrum of chlorophyll mutations like albina, xantha, chlorina and viridis could be recorded in M_2 generation, grown from the harvested seeds of M_1 in Chickpea. The frequency of these mutants revealed an increasing trend with an increasing concentration of all the mutagens in both the cultivars of chickpea. The frequency and spectrum of chlorophyll mutants induced by EMS, SA and Gamma rays' mutagens in Chickpea varieties BDNG-797 and PG 0408. High frequency of mutant in BDNG 797 were recorded in SA 0.04% treatment while in PG0408 highest frequency was observed while 0.06% SA treatments.

KEY WORDS

Induced mutation, Chlorophyll mutants, EMS, SA and Gamma rays, chickpea.

INTRODUCTION:

Chickpea belongs to family Leguminosae a view accepted by the majority of biologist[1]. However, some taxonomist follows Hutchinson's classification and according to this classification, chickpea belong to the family Fabaceae (Papilionaceae) of the order leguminales and class dicotyledonous. The binomial nomenclature of chickpea is *Cicer arietinum* L., where *cicer* is the genus and *arietinum* is the species.

Chickpea (*cicer arietinum* L.), acquires importance for cheap, vegetable protein, good source of carbohydrates, low cost of production and capacity for fixing atmospheric nitrogen. It plays pivotal role of supplying protein source in the vegetarian diet and is also called poor man's meat supplementary. Production and supply of cereals with high protein legume is potentially one of the best solutions to protein calorie malnutrition, particularly in India and other developing countries.

In recent years a lot of work has been undertaken on induced mutagenesis through physical and chemical mutagens with keen interest to know its impact on food security and malnutrition conditions. It has been clearly shown in a number of plant species that the effect induced, varies with the varying mutagens and with the variation in mutagen doses. Thus selecting a mutagen and its optimum dose for a genotype in any plant species is an important step in mutation breeding programme.

Inducing mutations provide beneficial variations for practical plant breeding purpose. These mutagens may cause genetic changes in an organism, break the linkage and produce many new promising traits for the improvement in crop plants. During the past seven decades more than 2252 mutant varieties have been officially released in the world. Many chemicals mutagens have been used for induction at useful mutants in a number of crops[2]. Adaptation of new techniques, as a dependable method of crop improvement, depends very much on the identification of more effective and efficient mutagens as well as on the improved methodology adopted to increase the spectrum of useful mutation in the oligonenic and polygenic traits.

The potential of induced mutations in widening the genetic diversity is now establishing in chickpea. The mutagenic effect is reflected in the segregation of chlorophyll mutants and it is also a good indicator to forecast the spectrum of genetic variability that can arise from the mutated sectors[3,4]. The present investigation evaluates the effect of different doses/concentrations of EMS, SA and Gamma rays mutagen on the frequency and spectrum of chlorophyll.

MATERIALS AND METHODS:

The genetically pure experimental seeds of Chickpea variety (BDNG – 797 and PG 0408) were procured from Mahatma Phule Agriculture University, Rahauri (Ahamadnagar) & Agriculture Research Station Badnapur (Jalna) Maharashtra. The physical mutagen Gamma rays and chemical mutagens EMS and SA were practiced for this experiment.

The seeds dried to reduce moisture content up to 10-12% (1000) each dose / concentration comprised of 1000 seeds. These seeds were irradiated with 10kR, 20kR & 30kR doses at gamma rays from 60 co source at Department of Biophysics, Government institute of science, Aurangabad (M.S.) a dose Rate of .234. kr/h.

The solutions of chemical mutagens namely Ethyl methane sulphonate (EMS) and sodium azide (SA) were prepared. The healthy seeds were presoaked in distilled water for 4 hours of room temperature followed by six hours treatment with Various Concentration such as 0.05%, 0.10% and 0.15% EMS and 0.02%,0.04% & 0.06% SA Mutagens. Then these seeds were thoroughly washing under running water. The 900 seeds from each treatment were sown in Randomized Blocked Design (RBD) with three replications along with control for rising M_1 generation during the month of October.

Recommended agronomic, (RBD) practices were employed for preparation at field sowing and subsequent management at the population. The M_1 selected plant seeds were collected from all the treatment and control they were used for raising the M_2 generation. Chlorophyll mutations were scored in the field when the seedling was 10-15 days old. The types chlorophyll mutation scored were *xantha, chlorina, viridis and albina*. The frequency of chlorophyll mutant was calculated.

RESELTS:

Chlorophyll mutations (Table: 1-2)

In the present investigation, a wide range of chlorophyll mutants were recorded in the M_2 population in all the mutagenic treatment. Chlorophyll mutants were identified using the criteria[5 & 6]. The chlorophyll mutants were of different types such as *xantha, chlorina, albino and viridis. Xantha* mutants show a bright yellow colour and deep golden yellow colouration. *Chlorina* mutants were yellowish green in colour, the *viridis* mutants showed dull light green colour and *albino* mutants displayed white colour.

The chlorophyll mutations increased with the increase in the dose and concentration of the mutagens among the three groups of mutagens. The highest percentage of chlorophyll mutants in both the cultivars followed by sodium azide (SA) in BDNG 797, the highest percentage mutants (14.87%) were exhibited by 0.06% SA treatment while in PG0408 it was also showed by 0.06% (SA) concentration (14.59%). The total percentage at mutants was high in chickpea variety BDNG 797 as compare of to PG 0408.

Spectrum of chlorophyll mutant: (Table.3-4)

In the present investigation *albina, xantha, viridis and chlorine* four types of chlorophyll mutations identified. The spectrums of chlorophyll mutants such as *chlorina*, *xantha, albino and viridis* grew well, got flowered, bore pods and survived till the maturity. But the plants having albino characters did not survive for more days, and they provided to be lethal.

Albina

In BDNG797, the *albina* mutants were recorded in each of the three treatments i.e EMS, SA and Gamma rays except 0.15% concentration of EMS and 0.04% of SA. With high percentage in treatment followed by 30 kR Gamma rays dose (0.92%). While in PG 0408, the mutants were recorded from all the treatments except 0.05% EMS treatment. The highest percentage of mutants followed by 0.06% of SA treatments (1.22%)

Xantha

The *xantha* mutants were recorded in all the three treatment of mutagens in both the cultivars of Chickpea. The high percentage of spectrum of chlorophyll mutant was recorded in 0.06 % of SA mutagens in both the varieties of Chickpea. In BDNG 797 the high percentage of chlorophyll spectrum was noticed 4.20%. While in variety PG 0408 the highest percentage of chlorophyll spectrum was 3.68%.

Viridis

The *viridis* mutants were recorded in the entire three treatment i.e EMS, SA and Gamma rays. The high percentage of *viridis* spectrum of chlorophyll mutants was observed in 0.06% of SA treatments (5.21%) in variety BDNG 797, while in variety PG 0408, the highest percentage of mutants observed (4.09%) in same mutagenic concentration.

Chlorina

The *chlorina* spectrum of chlorophyll mutants were recorded in all three mutagens with entire treatments in both the variety of Chickpea namely BDNG 797 and PG 0408. The high percentage of spectrum of chlorophyll mutants were observed in 0.06% treatments of SA mutagens. In BDNG 797 high percentage of spectrum of chlorophyll mutant recorded in 0.04% of SA treatments (5.48%). While in variety PG 0408, the mutants were recorded in 0.06% of SA treatment (5.59%).

Table: 1. Effect of mutagens on the frequency of chlorophyll mutant in M_2 generation in Chickpea. Variety: BDNG 797.

Treatment	Concentration /Dose	Total No. of seedling recorded	No. of mutant	Total mutation frequency (%)
Control	-	-	-	
	0.05	1033	61	5.91
EMS (%)	0.10	1033	69	6.70
	0.15	1260	102	8.90
	0.02	1550	176	11.32
SA (%)	0.04	1132	62	14.57
	0.06	1190	177	**14.87**
	10 kR	1380	71	5.14
Gamma rays	20 kR	1276	77	6.03
	30 kR	980	91	9.28

Table: 2. Effect of mutagens on the frequency of chlorophyll mutant in M_2 generation in Chickpea. Variety: PG 0408.

Treatment	Concentration /Dose	Total No. of seedling recorded	No. of mutant	Total mutation frequency (%)
Control	-	-	-	-
	0.05	1730	100	5.78
EMS (%)	0.10	1280	78	6.09
	0.15	1605	119	7.41
	0.02	1320	148	11.21
SA (%)	0.04	850	103	12.79
	0.06	733	107	**14.59**
	10 kR	1262	53	4.19
Gamma rays	20 kR	1385	74	5.34
	30 kR	1132	65	5.74

Table: 3. Effect of mutagens on the spectrum of chlorophyll mutant in M_2 generation in Chickpea. Variety: BDNG 797.

Treatment	Concentration /Dose	Relative % of Chlorophyll mutants.			
		Albino	*Xantha*	*Chlorina*	*Viridis*
Control	-	-	-	-	-
EMS (%)	0.05	0.48	1.25	2.42	1.74
	0.10	0.29	1.65	2.52	2.23
	0.15	-	2.38	2.94	2.78
SA (%)	0.02	0.32	3.29	4.13	3.61
	0.04	-	4.15	**5.48**	4.95
	0.06	0.50	4.20	4.87	5.21
Gamma rays	10 kR	0.57	1.52	1.66	1.59
	20 kR	0.78	1.57	1.96	1.72
	30 kR	0.92	2.55	3.06	2.76

Table: 4. Effect of mutagens on the spectrum of chlorophyll mutant in M_2 generation in Chickpea. Variety: PG 0408.

Treatment	Concentration /Dose	Relative % of Chlorophyll mutants			
		Albino	*Xantha*	*Chlorina*	*Viridis*
Control	-	-	-	-	-
EMS (%)	0.05	-	1.59	2.54	2.08
	0.10	0.70	1.56	2.03	1.80
	0.15	0.56	2.05	2.49	2.31
SA (%)	0.02	0.83	2.65	4.02	3.26
	0.04	0.59	3.29	4.59	3.65
	0.06	1.22	3.68	**5.59**	4.09
Gamma rays	10 kR	0.24	0.95	1.35	1.35
	20 kR	0.58	0.79	2.09	1.88
	30 kR	0.71	1.24	2.21	1.59

DISSCUSSION

Though the chlorophyll mutations do not yield viable seeds, they are useful in understanding different physical functions and pathological manifestations. They also help in the study of the effects of specific genes products in differentiations[7]. Mutations in genes responsible for chlorophyll synthesis bring outs deficiency of chlorophyll pigments. Chlorophyll mutations have been used as an index to evaluate the mutagenic potential of various physical and chemical mutagens in number of crop plants.

In the present study the frequency of chlorophyll mutants was high BDNG-797 compared to PG0408. This varietal difference with respect to the frequency of chlorophyll mutations may be attributed due to differences in radio sensitivity. However,[5] opined that varietal differences and variations in incidence of chlorophyll mutations is due to the differences in the number of genes controlling the chlorophyll development in different varieties. Similar results noted [6] in *Trigonella*. Recently,[1] in pigeon pea and[2] in fenugreek found that SA and EMS proved to be more efficient.

Differences in the effect of gamma rays and EMS on the frequency and spectrum of chlorophyll mutations can be attributed due to preferential action of EMS /gamma rays on genes for chlorophyll development located near the centromere. In the earlier studies also, the dose dependent increase in the frequency of chlorophyll mutations has been reported in a variety of crops that is soybean.[7], Urdbean.[8,9,10,11,12]

The frequency of chlorophyll mutation in M_2 generation can be considered as a reliable index for estimation the potency of mutagens. In several earlier studies, the most frequent type of chlorophyll mutant induced due to gamma rays was also found to be *albina or viridis*[13,]

[14,15]. It is thus obvious from the present study that *albina* in addition to *viridis or Xantha* is one of the frequent types of chlorophyll mutants induced by gamma rays, SA and EMS. While, comparing mutagens, it has been observed in several studies that chemical mutagens were more effective than radiation doses[5].

CONCLUSION

The frequency of chlorophyll mutation is useful in assessing the potency of mutagen and it is also an indicator of factor mutation and dependable index for evaluating genetic effects of mutagenic treatments. It helps study of specific gene products which responsible for chlorophyll synthesis. The spectrum of chlorophyll mutations became broader with increasing concentrations of the two mutagens in both the cultivars of chickpea. The different types of chlorophyll mutant obtained in the present study comprised: *xantha, chlorina, viridis* and *albino.*

Marked varietal differences were present in the expression of induction of chlorophyll mutations at different doses/concentrations of mutagens due to genetic differences existing among the two varieties.

It is, therefore, concluded that although 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 and number of economically useful mutants in the segregating generations.

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***Corresponding Author:**
Manisha G. Nagargoje*
Email: manisha.mukta21@gmail.com