ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 10, pp.21101-21104, October, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

# GENETIC VARIABILITY INDUCED BY ETHYL METHANE SULPHONATE AND SODIUM AZIDE IN CHLOROPHYLL MUTATION IN CHICKPEA (*CICER ARIETINUM* L.)

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ARTICLE INFO

*Article History:*
Received 07th July, 2015
Received in revised form
15th August, 2015
Accepted 21st September, 2015
Published online 20th October, 2015

*Key words:*
Chickpea, Chemical mutagens,
Chlorophyll mutation.

ABSTRACT

Chlorophyll mutations are considered as indicators of mutability, in present investigation a wide spectrum of chlorophyll mutations like *Xantha, Chlorina, Chloroxantha, Viridis* and *albino* could be recorded in M2 generation, grown from the harvested seeds of M1 in Chickpea (*Cicer arietinum* L.). The frequency of these mutants revealed an increasing trend with an increasing concentration of all the mutagens in both the cultivars of chickpea.



***Citation***: Navnath G. Kashid and Subhash B. More, 2015. "Genetic variability induced by ethyl methane Sulphonate and sodium Azide in chlorophyll mutation in chickpea (*Cicer arietinum* L.)", *International Journal of Current Research*, 7, (10), 21101-21104.

## INTRODUCTION

Chickpea is an important nutritious food legume which has high content of proteins, carbohydrates, calories, fats, vitamins and minerals. Pulses are commonly consumed with cereals and make good the deficiency of lysine in cereals, while cereals supply sufficient sulphur containing amino acids to supplement the deficiency in pulse proteins.

In general, pulses provide 20-30 % proteins, about 60% carbohydrates, 1-2.5 % fat and comprise good sources of vitamins like thiamine, riboflavin, nicotinic acid, ascorbic acid, carotene etc. The pulses are good sources of minerals and their total content varies from 3 to 4.5 % (Salunke, 1982). Charaka, the physician of ancient India, has identified a number of pulses as an important dietary component in disease management (Kunverba 1949). In fact pulses have also been recognized as part of functional foods and cheapest body building food. Pulses constitute a major source of a nutritive food for millions of people the world over for predominantly vegetarian population, especially for socio- economic groups.

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Mutation may use to correct undesirable characters or to get new genetic combination, which is desirable without changing the major part of total genetic setup. Different workers have been proved the practical utility of induced mutation. The idea of induced mutation artificially and using them for plant improvement has been clearly stated as early as 1901 by Hugo de vries.

The mutation breeders have visualized that the desirable mutant in different legumes and oil crops would be able to contribute effectively towards yield and protein content besides providing induced variation for diseases, insects and pest resistance.

## MATERIALS AND METHODS

In the present investigation two varieties of chickpea (*Cicer arietinum* L.) namely, BDN9-3 and PG-5, practiced. The seed material of chickpea cultivars obtained from Agricultural Research Station Badnapur, Dist: Jalna (Maharashtra) and MPKV, Rahuri, Dist: A. Nagar (Maharashtra) India, respectively. For further mutation breeding programme two chemical mutagens namely Ethyl methane sulphonate (EMS) and Sodium Azide (SA) were employed in the present investigation. Ethyl methane suphonate (CH3SO2O2C2H5) a monofunctional alkylating agent, with molecular weight of 124.16 manufactured by Sisco research laboratory, Mumbai and Sodium Azide (NaN3), with molecular weight of 65.01, manufactured by Spectrochem *pvt*. Ltd. Mumbai was used in the present work.

The treated and control plants were screened for the frequency and spectrum of chlorophyll mutation during seedling stage up to a period of 15 days and the pertinent values were calculated and the number of mutations per 100 plants of M2 generation was estimated according to the method of Gaul (1957). The classification and characterization of various chlorophyll mutations was done according to Gustafson (1940) and Blixt (1961), and the spectrum was recorded as (*Albino)* White in colour, (*Xantha)*Yellow to bright yellow in colour, (*chloroxantha)* greenish yellow in colour (*Chlorina)*Yellowish green in colour and (*Viridis)* Light green or yellow green in colour.

## RESULTS

### Chlorophyll mutation: (Table 1, 2, 3, 4)

In the present investigation, a wide range of chlorophyll mutants were recorded in the M2 population in all the mutagenic treatment. Chlorophyll mutants were identified using the criteria suggested by Gustafsson (1940) and Blixt (1961).

Chlorophyll mutations are considered as indicators of mutability. The chlorophyll mutants were of different types such as *xantha, chlorina, chloroxantha, albino and viridis. Xantha* mutants show a bright yellow colour and deep golden yellow colouration. *Chlorina* mutants were yellowish green in colour, the *chloroxantha* mutants were greenish yellow in colour, the *viridis* mutants showed dull light green colour and *albino* mutants displayed white colour.

The chlorophyll mutants such as *chlorina*, *xantha, chloroxantha* 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.

The frequency of chlorophyll mutants revealed an increasing trend with the increasing concentration of all the mutagens in both the cultivars. In case of EMS treatments the frequency of chlorophyll mutants in BDN 9-3 was 06% at 0.05%, 11.00% at 0.10% and 14.00% at 0.15%, while in PG-5 it ranged from 5% to 16%. In case of SA in BDN 9-3, the frequency of chlorophyll mutant ranged from 4% to 12% and in PG-5 it was between 4% to 15%. Of the varied chlorophyll mutants recorded, the *chlorina* and *chloroxantha* occurred in higher frequency while the albino revealed vary low frequency value in case of both the cultivars of chickpea.

**Table 1. Effects of mutagens on the frequency of chlorophyll mutants in M2 generation of chickpea, Variety: BDN 9-3**

| Treatment | Concentration % | Frequency of chlorophyll mutants % |
|---|---|---|
| Control | - | - |
| | 0.05 | 6 |
| EMS | 0.10 | 11 |
| | 0.15 | 14 |
| | 0.01 | 04 |
| SA | 0.02 | 09 |
| | 0.03 | 12 |

**Table 2. Effects of mutagens on the frequency of chlorophyll mutants in M2generation of chickpea, Variety: PG-5**

| Treatment | Concentration % | Frequency of chlorophyll mutants % |
|---|---|---|
| Control | - | - |
| | 0.05 | 5 |
| EMS | 0.10 | 12 |
| | 0.15 | 16 |
| | 0.01 | 04 |
| SA | 0.02 | 11 |
| | 0.03 | 15 |

**Table 3. Effects of mutagens on the spectrum of chlorophyll mutants in M2 generation of chickpea, Variety: BDN 9-3**

| Treatment | Concentration % | Frequency of individual mutants (%) | | | | |
|---|---|---|---|---|---|---|
| | | *Albino* | *Xantha* | *Chloroxantha* | *Chlorina* | *Viridis* |
| Control | - | - | - | - | - | - |
| | 0.05 | 0.7 | 3.2 | 18.5 | 15.1 | 10.2 |
| EMS | 0.10 | 1.2 | 6.4 | 21.4 | 28.6 | 12.4 |
| | 0.15 | 2.1 | 5.2 | 26.8 | 31.8 | 16.6 |
| | 0.01 | 0.4 | 2.8 | 14.2 | 12.4 | 8.3 |
| SA | 0.02 | 0.9 | 4.8 | 18.9 | 25.2 | 10.4 |
| | 0.03 | 1.8 | 6.3 | 23.5 | 28.3 | 13.5 |

**Table 4. Effects of mutagens on the spectrum of chlorophyll mutants in M2 generation of chickpea, Variety: PG-5**

| Treatment | Concentration % | Frequency of individual mutants (%) | | | | |
|---|---|---|---|---|---|---|
| | | *Albino* | *Xantha* | *Chloroxantha* | *Chlorina* | *Viridis* |
| Control | - | - | - | - | - | - |
| | 0.05 | - | 2.8 | 12.6 | 11.2 | 8.4 |
| EMS | 0.10 | 1.8 | 5.2 | 18.4 | 22.8 | 14.2 |
| | 0.15 | 3.2 | 6.8 | 24.2 | 28.4 | 12.8 |
| | 0.01 | 0.4 | 3.2 | 10.2 | 10.5 | 9.5 |
| SA | 0.02 | 1.2 | 4.9 | 14.8 | 20.6 | 11.4 |
| | 0.03 | 2.4 | 5.1 | 21.4 | 24.8 | 11.9 |

## DISCUSSION

### Chlorophyll mutations

The frequency of induced chlorophyll mutations in M2 generations has been considered as a reliable index for estimating the potency of mutagen due to greater accuracy in scoring (Gustafson, 1940). The chlorophyll mutations have been utilized not only as a major for assessing the effectiveness and efficiency of mutagens, but also as indicators to predict the size of vital mutations. This practice has been put into use for a number of mutagens, both the physical and chemical and their combinations (Gaul, 1965).

Though the chlorophyll mutations do not yield viable seeds, they are useful in understanding different physical functions and pathological manifestations (Miller, 1968). They also help in the study of the effects of specific genes products in differentiations (Robbelen, 1968). Mutations in genes responsible for chlorophyll synthesis bring outs deficiency of chlorophyll pigments. Most of the mutants are usually lethal but viable types are also known (Kothekar *et al.,* 1994). In the present study 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, chloroxantho, viridis* and *albino.*

The chlorophyll mutation frequency in the present investigation increased with the increasing concentrations of mutagen. This conformed to the results of Ehrenberg (1960), Micke (1961), Jana (1963), Blixt (1964), Konzak *et al.* (1965), Sudharrani (1990), Gautam *et al*. (1992) and Rayyan (1995). In the present study EMS treatments generated more chlorophyll mutants than SA in both the cultivars of chickpea. This confirmed the finding of Gaikwad (2002) in lentil where EMS was found to be the most efficient mutagen as compared with gamma rays and HA.

Sjodin (1962), Zannone (1965), Wellensiek (1965) and Monti (1968) have reported a higher frequency of chlorophyll mutations following treatments with chemical mutagens rather than radiations in several legumes. Monti (1968) reported that DES was 3-4 times more efficient in blackgram than x-rays in peas. Singh and Chaturvedi (1987) observed that the chlorophyll mutations showed remarkable differences between the spectrum induced by alkylating agents (EMS and NMU) and radiations (gamma rays). According to Swaminathan (1964) chlorophyll development seems to be controlled by many genes located on different chromosome. A variation in the number of loci (125-250) involved in chlorophyll synthesis has been proposed by Gustafsson (1963). Most of the mutations affecting these loci are inherited as monogenic (Nilan 1956, and Pereau- Leroy 1968).

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