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Induced mutagenesis in Green gram (*Vigna radiata* (L.) Wilczek)

V. Prabakaran[1], P. Manivel[2], S. Parvathi[3], S. Palanivel[*3]

[1,2]Research Scholar, PG and Research Department of Botany, Government Arts College (Autonomous), (Affiliated to Bharathidasan University, Trichy), Tiruchirappalli, Karur, Tamil Nadu, India

*[*3]PG and Research Department of Botany, Government Arts College (Autonomous), Tiruchirappalli, Karur, Tamil Nadu, India*

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Abstract

Induced mutagenesis was carried out in an important protein rich pulse crop (*Vigna radiata* (L.) Wilczek). The seeds of green gram variety Co-6 were treated with different concentrations of sodium azide. The mutagen treated seeds were sown in the field to observe M_1 characteristics. The sodium azide treated seeds were subjected to amino acid analysis. Totally 19 amino acids were recorded in control and sodium azide treated samples. In the process of sodium azide treatments a few amino acids were increased and some amino acids were decreased than control. The M_1 parameters such as germination and survival percentage, plant height, days taken for flowering, number of pods/plant, length of pods, number of seeds/pod and hundred seeds weight were decreased with increasing concentrations sodium azide and all the growth parameters showed negative trend when compared to control. The M_1 seeds were collected separately based on concentrations of sodium azide and stored for raising next generation after the harvest. The M_1 seeds were sown in the field to raise M_2 generation, and in M_2 population, the different types of chlorophyll and viable mutants were noticed, such as chlorina, xantha, viridis, and viable mutants such as tall, dwarf, leaf, pod and early flowering mutants were noticed in various treatments of sodium azide. In addition with chlorophyll and viable mutants several initial leaflet modifications like trifoliate, tetrafoliate and pentafoliate leaflets had been observed in mutagenic treatment with sodium azide. The present study is a basis for evolving mutant varieties in green gram with altered agronomic traits.

***Corresponding Author:** S. Palanivel ✉ ssspvrm@gmail.com

Introduction

Green gram or mung bean (*Vigna radiata* (L.) Wilczek) is one of the most important pulse crops in India and cultivated in different parts of the world. Protein rich edible seeds, sprouts rich in vitamins and amino acids are used directly and apart from this the crop is widely used as forage. However, the productivity and quality of the grain is severely reduced due to different stress factors in general. Despite its great economic importance a little information regarding its degree of stress tolerance is available through conventional studies, although yield losses are considerable when subjected to different stress conditions (Kaviraj *et al*., 2006).

Several biotic and abiotic factors as well as low genetic variability are supposed to be responsible for lowering the production of this important crop. During different stages of growing seasons, the loss exceed more than 50% due to incidence of many pests and diseases (Poehlman *et al*., 1991).

Induced mutagenesis is one of the traditional breeding methods in plant breeding. It is related with various fields like, morphology, cytogenetic, biotechnology and molecular biology etc. (Acharya *et al*., 2006). Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars of cereals, fruits and other crops (Lee *et al*., 2002). These mutations provide beneficial variations for practical plant breeding purpose. In the past seven decades, more thousands of mutant varieties have been officially released in the world (Maluszynski *et al*., 2000).

Sodium azide (SA-NaN3) is an ionic compound and its mutagenicity is interceded through a natural metabolite (undifferentiated from L-azidoalanine) of the azide compound produced by O-acetylserinesulfhydrylase catalyst (Gruszka *et al*., 2012). It is a chemical mutagen and it's one of the most useful mutagens in crop plants. The mutagenesis is mediated through the production of an organic metabolic of azide compound. This metabolic enters into the nucleus, interacts to DNA and creates point mutation in the genome. Several factors influenced the effect of mutagens such as properties of mutagens, duration of treatment, pH, pre and post treatment, temperature and oxygen concentrations etc. (Gehan *et al*., 2011).

The mutant plants formed by the application of sodium azide are able to withstand a range of unfavorable conditions and have enhanced yields, improved stress tolerance, longer shelf life and reduced agronomic input in comparison to a normal plant (Ahloowalia *et al*., 2002).

Like this, several authors carried out induced mutagenic studies in [*Vigna radiata* (L.) Wilczek] using physical and chemical mutagenic agents. (Wani *et al.,* 2017; Deswanjee *et al.,* 2018; Sofia *et al.,* 2020; Das *et al.,* 2020; Amol *et al*., 2021).

The production of new cultivar with enhanced amount of nutrients, tolerance to drought and salinity is still needed for this important legume crop. The main objective of the present part of the research work is to find out the effect of sodium azide on M_1 and M_2 generation of *Vigna radiata* (L.) Wilczek]. It is useful to carry out mutation breeding studies to obtain mutant varieties.

Material and methods

Seed material

Mature, healthy and uniform seeds of Green gram variety Co-6 was obtained from Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India is used as an experimental material to carry out the mutagenic studies using sodium azide as a chemical mutagen. The field experiments were conducted for various growth and yield parameters in M_1 generation and chlorophyll and viable mutants were recorded in M_2 generation.

Preparation of phosphate buffer

Solution 1:

Take 0.31 g of Na2PO4 and add 100 ml of distilled water to make up the solution 1.

Solution 2:

Then take 1.09 g of Na2HPO4 and add 100 ml of distilled water to make up the solution 2. Keep the solution in refrigerator for 30 min. After that both the solutions were kept for 30 min in room temperature. Then take 99 ml of solution-1 and 1 ml of solution-2 makeup to 1000 ml using dH2O. Finally the solution was set into 3 pH range.

Treatment with Sodium azide

For chemical mutagen treatment, (SA) the green gram seeds were placed in perforated polyethylene bags (with 3 replica each containing 50 seeds in separate polyethylene bags) and presoaked in distilled water for 6 hrs. The different concentration of sodium azide (10 to 50.0 mm) was prepared using phosphate buffer. Then the presoaked seeds were immersed in various concentrations of SA for 6 hrs. After mutagenic treatment the seeds were carefully washed with distilled water to remove the traces of SA present on the surface of the seeds. After treatments with sodium azide the seeds were immediately sown in the field to rise M_1 generation.

Amino acid analysis

The seeds of green gram cultivar Co-6 were treated with different concentrations of sodium azide for 6 hours, after pre-soaked with 6 hours in dH_2O. The treated seeds were dried in shade for few days and become powdered. The SA treated and control samples were subjected to HPTLC analysis for amino acids. The Amino acid analysis was carried out using HPTLC in control and sodium azide treated seeds of green gram and this method was suggested by (Harborne, 1998).

Results and discussion

Amino acid analysis

In this amino acid analysis, there were totally 19 amino acids recorded in control as well as in SA treated samples but their contents were different based on the concentrations of SA. The aminoacids like aspargine, histidine, proline, phenylalanine and lysine were increased in their content from 10mM to 30 mM but decreased in 40 and 50 mM of SA.

The other amino acids like serine, arginine, glutamine and valine had increased up to 20 mm SA. After that there was a gradual decrease in 30, 40 and 50 mm SA treatments. Totally 9 amino acids decreasing and increasing trend based on SA treatment and the remaining 10 amino acids such as aspartic acid, lycine, alanine, glutamic acid, threonine, isoleucine, methionine, tyrosine, leucine and cytidine had found slow and gradual reduction in their content from 10 to 50 mM SA when compared to the control. As like, the present study, on the effect of ethyl methane sulphonate on M_1 generation of groundnut and found out the distribution pattern of amino acids were highly influenced by EMS (Muniappan *et al.*, 2012). (All the amino acid profiling shown in Table 1 and Fig. 1)

Table 1. Effect of sodium azide on amino acid content in seeds of green gram (*Vigna radiata* (L.) Wilczek).

Name of the protein	Concentration in µg					
	Con	10mM	20mM	30Mm	40mM	50mM
Lysine	0.73	0.70	0.70	0.71	0.69	0.72
Proline	0.96	0.92	0.91	0.89	0.76	0.66
Histidine	0.39	0.37	0.37	0.33	0.30	0.26
Glycine	1.07	1.02	1.01	0.99	1.09	0.99
Asparagine	0.89	0.72	0.80	0.80	0.77	0.69
Serine	2.82	2.44	2.69	2.69	2.60	3.01
Arginine	1.80	1.55	1.71	1.72	1.66	1.92
Glutamine	1.07	1.04	1.04	1.03	1.10	1.09
Cystine	0.89	0.87	0.87	0.84	0.92	0.92
Aspartic acid	1.86	1.82	1.80	1.86	1.92	1.90
Alanine	1.09	1.07	1.06	1.00	1.13	1.12
Glutamic acid	1.21	1.22	1.23	1.25	1.35	1.33
Threonine	1.05	1.06	1.07	1.09	1.18	1.16
Valine	1.11	1.11	1.12	1.14	1.23	1.22
Isoleusine	0.46	0.44	0.41	0.48	0.63	0.59
Methionine	1.40	1.32	1.36	1.39	1.59	1.57
Tyrosine	1.81	1.70	1.69	1.79	2.05	2.03
Leucine	0.94	0.89	0.88	0.94	1.07	1.06
Phenyl alanine	0.85	0.80	0.79	0.89	0.96	0.96

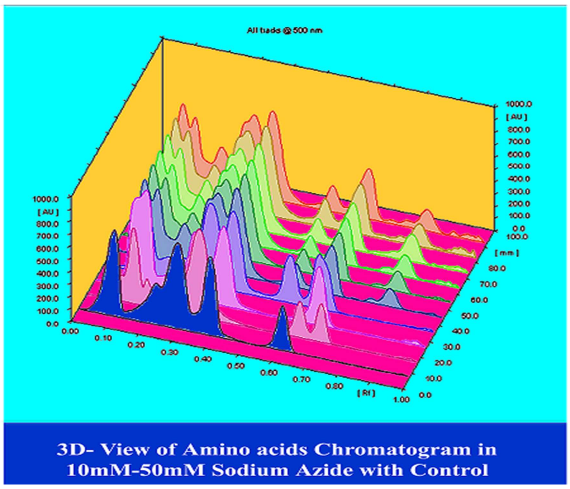

Fig. 1. Amino acid bands scanned at 500 nm in (*Vigna radiata* (L.) Wilczek).

Growth parameters

The M_1 characteristics such as percentage of seed germination, survival percentage, plant height (on 30^{th} day), number of pods, pod length, number seeds per pod, 100 seeds weight, total plant height at the time of harvest were calculated.

The germination percentage was decreased with increasing concentrations of sodium azide. The percentage of seed germination in control was 88% and it was decreased to 30% in 50 mM SA treatment but there was a steady decline in percentage of germination starting from 10 mM SA treatment.

In lower concentrations of SA the reduction in germination was lower but it was increased with increasing concentrations. The percentage of survival also decreased in various treatments of sodium azide when compared to control and the percentage of survival on 30th day was 98% in control and 70% in 50mm SA treatment with on increased concentrations of SA the percentage of survival decreased gradually from 95% to 70%.

The reduction in seed germination and seedling survival in M_1 generation due to sodium azide had been observed in mungbean (Awan *et al.*, 1980, Mahna *et al.*, 1989, Traiq *et al.*, 2007, Mahto *et al.*, 2018, Vikhe *et al.*, 2020, and Sofia *et al.*, 2020). The reduction in survival percentage of M1 generation was decreased with increasing in concentrations of mutagen was also confirmed with the earlier reports in soybean (Pepo *et al.*, 1989) and mung bean (Pavadai *et al.*, 2009). The reduction in survival percentage of the treated population could be due to disturbed physiological activity or chromosome damage leading to mitotic arrest (Sato *et al.*, 1967).

The plant height was measured on 30th day, the influence of sodium azide on plant height was decreased positively from 10mm to 50mM SA when compared to control. The days taken for flowering were delayed with increasing concentrations of sodium azide. In control 50 % flowering was 37 days but it was increased by SA treatment. In 10 mm SA the days taken for 50 % flowering was 40 days and it was increased up to 50 days in 50 mM SA treatment.

The higher concentrations of SA delayed the floral initiation. The percentage of germination, survival rate, plant height (30th day) were showed negative trend with increasing concentrations of SA.

Such a decrease in seedling height might be happened due to inhibition of cell division, due to auxin destruction, changes in ascorbic acid content and physiological injury in the seeds (Ignacimuthu *et al.*, 1988). Similar type of mutagenic sensitivity in mungbean genotype was also reported earlier (Kuldeepsingh, 2013 and Rukesh *et al.*, 2017).

Yield characteristics

The yield characters such as pod yield was increased up to 30 mm SA than control and showed negative tendency in 40 and 50 mm SA treatments. In control the number of pods per cluster was 5.54, it was increased up to 7.50 in 20 mm SA and reduced to 3.80 in 50 mm SA. The maximum pod length was 7.90 cm noticed in 10 mm SA treatment. The progressive increase in length of pod was recorded up to 30 mm SA, but there was a reduction in 40 and 50 mm concentrations. There was a variation in the number of seeds per pod in control and SA treated populations.

The number of seeds ranged from 6.63 in control and increased up to 6.93 in 10 mM, 7.82 in 20 mm, 7.18 in 30 mm (increased over control) but 6.51 and 5.21 in 40 and 50 mM SA. The numbers of seeds were exceeded over control up to 30 mM in SA treatments.

The seed yield was increased only in lower doses of mutagens of EMS, SA and gamma rays were supported by (Singh *et al.*, 2009). The each 100 seeds from control and treatment with SA were collected randomly and treatment weight. The maximum seed weight (3.80 g) in10mm SA treatment and lowest 2.80 g was observed in 50 mm SA treatment. The weight the 100 seeds were increased up to 30 mm SA over control and reduced in 40 and 50 mm SA (All the growth and yield parameters shown in Table 2).

M_2 generation-chlorophyll mutants

The spectrum of induced chlorophyll mutants of sodium azide was studied in M_2 generation of green gram. The different types of chlorophyll mutants were noticed in various concentrations of sodium azide. The occurrence of albino, maculata and albino viridis were totally absent in all the treatments of sodium azide.

Table 2. Effect of sodium azide on M_1 attributes in green gram (*Vigna radiata* (L.) Wilczek).

Treatment	Germination percentage	Survival percentage	Plant height (30^{th}day) (cm)	Days taken for flowering	Number of pods/ Cluster	Length of pods (cm)	Number of seeds/pod	100 Seeds weight (g)
Control	88.00±7.27	98.00±0.94	19.42±0.50	37.52±0.34	5.54±1.22	7.13±5.63	6.63±0.62	3.61±0.48
10mM	75.00±3.60	95.00±1.23	16.56±0.43	40.44±0.18	7.21±0.64	7.50±0.21	6.93±0.17	3.80±0.09
20mM	59.00±6.80	90.00±1.14	13.25±0.39	43.55±0.41	7.50±0.95	7.90±6.42	7.82±0.35	4.37±0.17
30mM	44.00±4.00	86.00±0.54	11.58±0.34	45.64±0.10	6.30±0.55	7.20±0.31	7.18±0.34	4.10±0.27
40mM	36.00±1.52	80.00±0.94	9.38±0.26	47.47±0.11	4.90±1.40	6.50±0.72	6.51±0.62	3.33±0.13
50mM	30.00±4.35	70.00±1.24	6.91±0.46	50.00±0.03	3.80±0.40	4.70±0.42	5.21±0.21	2.80±0.17

The other chlorophyll mutants such as chlorina, viridis and xantha were recorded in various frequencies. (All the chlorophyll mutants were shown in table 2). In the present study, the different types of chlorophyll mutants were noticed in M_2 generation. The chlorophyll mutants reported in our study was confirmed with several earlier reports and the three different types of chlorophyll mutants observed in M_2 generation by using EMS, MMS and SA (Khan *et al.*, 1993). There are some important of chlorophyll mutants such as albino, xantha, chlorina and viridis induced by gamma rays in (*Vigna radiata* (L.) Wilczek) (Sanjai Gandhi *et al.*, 2014). The frequency of chlorophyll had been dependent and increased by increasing concentrations of SA and the wide spectrum of chlorophyll mutants in M_2 generation of mungbean following mutagenesis with MMS, HZ and SA as per the study of Mohammed Rafig Wani, 2020 (Table 3 and Fig. 2).

Table 3. Effect of sodium azide on inducing chlorophyll mutants in M_2 generation (*Vigna radiata* (L.) Wilczek).

Treatment	Total No. of M_2 plants	Types of chlorophyll mutants				
		Xantha	Chlorina	Viridis	Total number of chlorophyll mutants	Percentage of chlorophyll mutants
Control	560	-	-	-	-	-
10 mM	765	1	2	3	6	1.24
20 mM	643	2	5	4	11	1.92
30 mM	610	4	8	6	18	2.95
40 mM	570	5	14	8	27	4.19
50 mM	483	6	20	11	37	4.83

M_2 generation-viable mutants

In M_2 generation, the different types of viable mutants were observed in various treatments of sodium azide such as tall mutant, dwarf mutant, leaf mutant, early flowering mutant, late flowering mutant and mutant with increased pod length were recorded in M_2 population. Among the viable mutants, tall mutant (taller than control) was more prominent than others followed by dwarf mutant. The tall mutants were noticed in lower concentrations of sodium azide. In this study, early flowering mutants had been reported. The same trend was supported by (PriyaranhanTah, 2006).

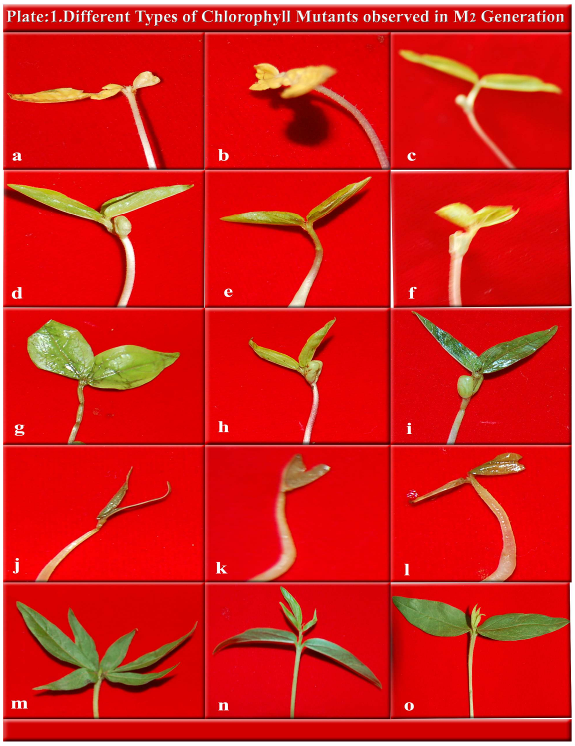

Fig 2. Different types of chlorophyll mutants observed in M_2 generation.

a, b, c- Albino, d, e, f- Xanthag, h, i- Chlorino and j, k, l, m, n, o- Viridis

The mutants like compact, dwarf, early, large pod size and synchronous maturity mutants in two mungbean varieties 'PS 16' and Sona with the 10 to 60 kR gamma radiation and 0.1% to 0.4% EMS treatments (Anandkumar *et al.*, 2009). Some results also observed several types of viable mutants in mungbean (Auti, 2012).

The long pod mutants in cluster bean by the treatment with EMS, gamma rays and their combined treatment (Singh *et al.*, 1986). In this study, the initiation of leaf morphology with tetrafoliate, pentafoliate leaves and bilobed, trilobed and highly enlarged leaflets also induced by sodium azide. In the present part of research work, several leaflet modifications were noticed in sodium azide populations. Several authors confirming our study (Dahiya, 1973 and Singh *et al.*, 1981) and in mung bean (Khalil *et al.*, 1987). The bifoliate, tetrafoliate and pentafoliate leaves in mungbean with EMS by (Singh *et al.*, 1982 and Apparao *et al.*, 2005) (Table 4 and Fig. 3).

Table 4. Effect of sodium azide on including viable mutants in M_2 generation (*Vigna radiata* (L.) Wilczek).

Treatment	Total No. of M_2 plants	Types of viable mutants						Percentage of M_2 mutants
		Tall mutant	Dwarf mutant	Leaf mutant	Early flowering	Late flowering	Pod mutants	
Control	560	-	-	-	-	-	-	-
10 mM	765	10	2	1	4	-	8	3.26
20 mM	643	11	6	3	12	-	14	7.15
30 mM	610	31	10	4	-	-	-	7.37
40 mM	570	-	16	7	-	10	-	5.78
50 mM	483	-	35	11	-	16	-	12.83

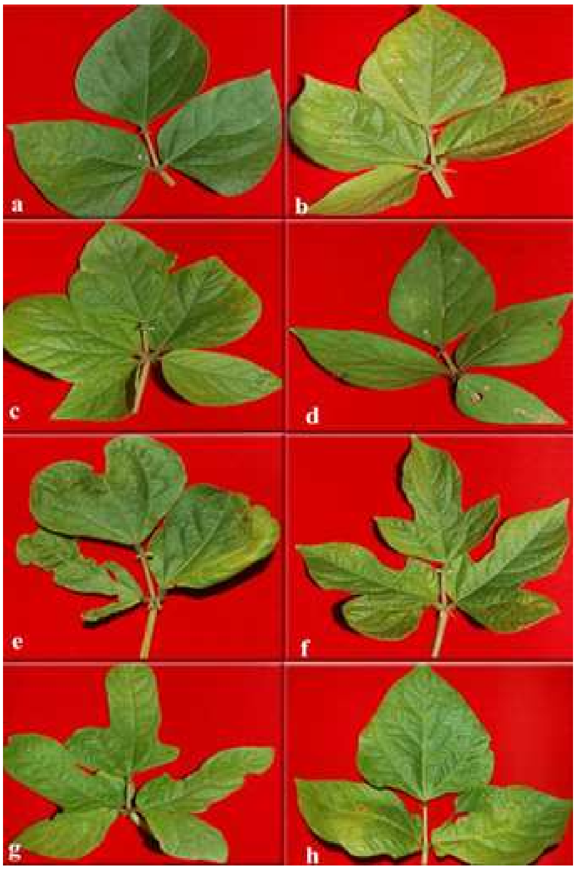

Fig 3. Different types of leaflet modifications observed in M2 generation on (*Vigna radiata* (L.) Wilczek) a. Normal trifoliate leaflet b. Tetra foliate c. Penta foliate, d-h. Variations in leaf modification.

Conclusion

Induced mutagenesis is a powerful tool for enhancing variability in several crop plants. In recent years induced mutations have also been gaining much importance in molecular biology for identifying the structure and functions of the gene. By using sodium azide as a chemical mutagen in green gram is useful to evolve mutants with improved characteristics.

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