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Induced chlorophyll and morphological mutations in Bambara Groundnut (*Vigna subterranea* (L.) Verdc.)

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ABSTRACT

The present study was conducted at K-block, University of Agricultural Sciences, Bengaluru. The experimental material for the present investigation consists of two varieties of Bambara groundnut having good agronomic base belonging to different accessions viz., SB-42 (light brown, round shape and white hilum) and S165A (dark brownish, dark spotted surface and oval shape). Five different concentrations of EMS 0.1, 0.2, 0.3, 0.4 and 0.5 per cent were used to treat the seeds. After the treatment, the seeds were sown in the field along with control (untreated seeds) to raise M_1 generation. The M_2 generation was raised during 2014 *summer* season by pooling all the seeds of M_1 plants on plant to row basis along with control plants. In both the varieties of Bambara groundnut, the mutagenic effectiveness and efficiency of mutagenic treatments varied not only between the two varieties but also between the treatments. A concentration of 0.3 per cent EMS was more effective than the other concentrations of EMS. Hence 0.3 per cent EMS has been regarded as LD50 for inducing useful mutations.

Key words: *Bambara groundnut, Induced mutation, EMS, Chlorophyll mutation, Morphological Mutation*

Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdc.), member of the family Fabaceae is a self-pollinating diploid annual legume crop with a chromosome number of 2n=22. It is an important food grain grown widely in semi-arid Africa and is closely related to cowpea (*Vigna unguiculata*) with which it shares its origin of genetic diversity. In Africa, Bambara groundnut is the third most important legume after groundnut and cowpea (Sellschope, 1962). *Vigna subterranea* is an extreme inbreeder; an autogamous crop with flower that are cleistogamous in nature (Uguru and Agwatu, 2006), which gives rise to high percentage selfing since the floral structure is perfect resulting in extreme inbreeding. For effective selection in any enhancement programme, genetic variation must exist.

Radiation and other chemical mutagens have been used to induce variability in crop plants (Ahloowalia *et al.,* 2004). Generally, EMS causes alkylation of guanine bases (G) leading to mispairing or mismatch pairing in the DNA of a treated organism. Under these conditions, an alkylated G pairs with T in place of C, causing a G/C to A/T transition in the backbone of the DNA (Henikoff and Comai, 2003). It can cause allelic mutations, small deletions and other chromosomal rearrangements. These mutations can be used to activate morphometric and reproductive changes in plants; further selection of mutant plants through a number of generations resulting in introduction of new traits into a treated population (Narottam Acharya *et al.,* 2007).

To improve upon the productivity of Bambara groundnut, strategies like genetic recombination and selection, induced mutation and appropriate biotechnological approaches are some of the techniques that could be used. Mutation breeding thus plays an important role in genetic improvement of Bambara groundnut. In this view objective was framed to assess mutagenic efficiency on morphological traits of Bambara groundnut using Ethyl methane sulfonate.

Materials and Methods

The present study was carried out at K-block, University of Agricultural Sciences, Bengaluru. The M1 generation was raised during kharif season of the year 2013 and M2generation was raised during summer season of the year 2014. The experimental material for the present investigation consisted of two varieties of Bambara groundnut having good agronomic base belonging to different accessions viz., SB-42 (light brown, round shape and white hilum) and S165A (dark brownish, dark spotted surface and oval shape).

Two hundred well matured healthy and uniform sized non-dormant seeds of each variety of Bambara groundnut were used for the chemical treatment. Five different concentrations of EMS 0.1, 0.2, 0.3, 0.4 and 0.5 per cent were used to treat the seeds. The solution of EMS was prepared corresponding to the required concentrations using double distilled water. The pH of aqueous solution was adjusted at 8.5 by using 0.2M solution of sodium tetra borate (Borax). The volume of solution prepared was about three times of volume of seeds. The seeds were presoaked in double distilled water for five hours at room temperature (28 ± 2ºC) prior to the treatment. After pre-soaking the excess of moisture in the seeds was then removed by filter paper. Seeds were then soaked in freshly prepared aqueous solution of different doses EMS for six hours at room temperature (28 ± 2ºC) with intermittent shaking after every hour. After the treatment, the seeds were washed thoroughly with double distilled water for eight to ten times and sown in the field along with control (untreated seeds) to raise M_1 generation. Pre-soaked seeds in only double distilled water for six hours were used as control. The M_2 generation was raised during 2014 summer season by pooling all the seeds of M_1 plants on plant to row basis along with control plants. The following observations were recorded in M_2 generation.

Chlorophyll variants: The chlorophyll variants were carefully scored on M_2 plants. Different types of chlorophyll variants are described below:

Albina: White leaves of seedlings and relatively smaller than the normal seedlings of same age. These variants survived for about a week.

Chlorina: Yellowish green, seedlings survived for 15 days.

Xantha: Straw yellow seedlings with normal growth in the beginning but started withering after 10 days, died within 15 days.

Viridis: Seedlings showed dark green colour of leaves. The plants were slow growing and had a low seed yield.

The variants that survived were observed periodically from the seedling stage to maturity in M_2 generation. The variants were described and classified based on the nature of deviations they showed from the parent. The variants were observed and recorded for plant height (dwarf and tall), growth habit (compact and bushy), leaf modification (narrow and broad), and maturity (early and late). The morphological mutants were labeled and harvested separately and their frequency was estimated on M_2 plant basis.

Results and Discussion

Effectiveness and efficiency of EMS in SB-42 and S-165A

Mutagenic effectiveness relates to the concentration of mutagen to the mutational events, while mutagenic efficiency indicates the extent of genetic damage recorded in M_2 generation in relation to the biological damage caused in M_1 (Konzak *et al.*, 1965). Mutagenic effectiveness and efficiency were estimated on the basis of the frequency of progenies segregating for chlorophyll mutations. In both the varieties of Bambara groundnut, the mutagenic effectiveness and efficiency of mutagenic treatments varied not only between the two varieties but also between the treatments.

Chlorophyll mutations represented in Table 1 and 2 indicate dose dependent increase in the chlorophyll mutation frequency in both varieties of Bambara groundnut. These results are in accordance with the reports of Kumar *et al*. (2009) in cowpea. However, at 0.3 per cent EMS, a higher frequency of chlorophyll mutation has been observed. Of the four types of chlorophyll mutants recorded in M_2 generation, chlorina followed by albina types were predominant in the two varieties. Vanniarajan *et al.* (1993) in *Vigna mungo* reported that the EMS produced a high frequency of chlorina type of chlorophyll mutants. At 0.3 per cent concentration of EMS a higher frequency of chlorophyll mutations was recorded than in the other treatments. Occurrence of chlorina mutants has been attributed to different causes such as impaired chlorophyll biosynthesis, further degradation of chlorophyll and bleaching due to deficiency of carotenoids. The frequency of albina mutants was more than the xantha and viridis. EMS is supposed to be specific to certain chromosomal regions containing genes for chlorophyll development and has been reported to induce high frequency of chlorophyll mutations.

A higher number of mutant seedlings were recorded in all treatments of EMS in SB-42 variety compared to that of the S-165A variety is an indication of the differential response of these two varieties to the mutagen used. Genetic differences even of a single gene induce significant changes in mutagen sensitivity which influences not only the rate but also the spectrum of recoverable mutations. Despite the fact that different frequencies of similar mutations (Chlorina, Xantha, Albina and Viridis) are induced by different mutagens, the chief limiting factor in the induction and recovery of mutations is the genetic constitution of the experimental material.

A concentration of 0.3 per cent EMS was more effective than the other concentrations of EMS. Hence 0.3 per cent EMS has been regarded as LD50 for inducing useful mutations these results are in accordance with Singh (2001) in cowpea, Deepalakshmi and Ananda Kumar (2003) in black gram, Singh *et al.* (2005) in mung bean, Singh *et al.* (2006) in cowpea, Sharma (2006) in black gram, Dhanavel *et al.* (2008) in cowpea, Singh *et al.* (2008) in mungbean, Senapati and Misra, (2008) in black gram, Girija *et al.* (2009) in cowpea, Girija and Dhanavel, (2009) in cowpea, Parmhansh *et al.* (2009) in mung bean, Tripathy (2009) in mung bean, Arulbalachandran and Mullainathan (2009) in black gram, Thilagavathi and Mullainathan (2009) in black gram, Wani Aijaz (2011) in mungbean, Kozgar *et al.* (2011) in *Vigna* and Vairam *et al.* (2014) in mung bean.

Effect of EMS on morphological mutations in M_2 generation

The present study has proved fruitful in inducing a wide range of morphological mutations (Table 3 and 4). Although some of them proved uneconomical, some mutants recorded can be utilized as a source of many beneficial genes in cross breeding programmes or for the improvement of some quantitative traits like yield. Various workers suggested that such mutants might be either a result of pleiotropic effects of mutated genes or chromosomal aberrations or gene mutations. Several workers like

Table 1. Spectrum of chlorophyll variants in M2 generation of SB-42

EMS concentration in per cent	Number of variants					Frequency	Efficiency	Effectiveness
	Albina	Chlorina	Xantha	Viridis	Total			
0.1	0	0	0	1	1	0.30	0.02	0.04
0.2	1	2	1	1	5	1.17	0.07	0.06
0.3	10	12	8	6	36	5.18	0.24	0.13
0.4	5	2	2	1	10	4.65	0.20	0.08
0.5	2	2	1	1	6	2.01	0.07	0.02

Table 2. Spectrum of chlorophyll variants in M2 generation of S-165A

EMS concentration in per cent	Number of variants					Frequency	Efficiency	Effectiveness
	Albina	Chlorina	Xantha	Viridis	Total			
0.1	0	0	0	0	0	0.00	0.00	0.00
0.2	0	0	1	2	2	0.88	0.04	0.03
0.3	9	11	6	31	31	5.53	0.18	0.10
0.4	3	5	2	11	11	5.09	0.15	0.06
0.5	1	1	1	1	4	2.50	0.06	0.02

Kumar *et al.* (2009) in cowpea, Sagade and Apparao, (2011) in urdbean, Devmani Bind and Dwivedi, (2014) in cowpea have reported that mutations like tall, dwarf and bushy were found. Morphological mutants, isolated in M_2 generation, differed not only in the two varieties of Bambara groundnut but also within variety in different mutagenic treatments, suggesting that the varieties responded differently to the dose and type of mutagens employed. Based on morphological mutation frequency, SB- 42(126 mutants) proved to be the more sensitive variety, than S-165A (39 mutants).

Table 3. Spectrum of viable variants in M2 generation of Bambara groundnut genotype SB-42

Types of variants	Concentrations of EMS in per cent				
	0.1	0.2	0.3	0.4	0.5
Plant height					
Tall	2	2	6	1	1
Dwarf	1	3	4	2	1
Growth habit					
Compact	2	1	3	0	0
Bushy	1	3	3	2	1
Foliage					
Narrow	1	1	16	2	1
Broad	1	1	14	1	1
Maturity					
Early	0	0	4	0	0
Late	0	0	0	1	0
Double seeded	2	8	14	4	6
Total	10	19	64	13	11
M_2 Plants	331	429	695	215	298
Frequency	3.02	4.43	9.21	6.05	3.69
Efficiency	0.24	0.26	0.42	0.25	0.13
Effectiveness	5.04	3.69	5.12	2.52	1.23

Table 4. Spectrum of viable variants in M2 generation of Bambara groundnut genotype S-165A

Types of variants	Concentrations of EMS in per cent				
	0.1	0.2	0.3	0.4	0.5
Plant height					
Tall	1	1	3	1	1
Dwarf	0	0	1	0	0
Growth habit					
Compact	0	0	1	0	0
Bushy	1	1	4	1	1
Foliage					
Narrow	0	0	5	0	0
Broad	0	0	10	0	0
Maturity					
Early	0	0	1	0	0
Late	0	0	0	0	0
Double seeded	0	0	5	1	0
Total	2	2	30	3	2
M_2 Plants	3	226	561	216	160
Frequency	0.62	0.88	5.35	1.39	1.25
Efficiency	0.04	0.04	0.17	0.04	0.03
Effectiveness	0.06	0.03	0.10	0.02	0.01

In both the varieties, among the all treatments 0.3 per cent of EMS produced maximum morphological mutation frequency followed by 0.4 per cent EMS. This is in agreement with earlier reports of Kumar *et al.* (2009) in cowpea, Sagade and Apparao, (2011) in urdbean, Devmani Bind and Dwivedi, (2014) in cowpea. Relative differences in the mutability of genes for different traits have been observed, as some of the mutant types appeared with higher frequencies than other mutants. For instance, plant height and leaf mutants appeared more frequently than growth habit, growth period and yield. The more frequent induction of certain mutation types by a particular mutagen may be attributed to the fact that the genes for these traits are probably more responsive to different mutagens with different modes of action. Nilan, (1967) concluded that different concentrations of mutagens and treatment procedures may also change the relative proportion of different mutation types.

In comparison with the control, plant height was reduced to different magnitudes in dwarf mutants and the shortest height was recorded in SB-42 genotype at 0.3 per cent of concentration. Such reduction in plant height was mainly due to short intermodal distances manifested in dwarf mutant. Rai and Hanna, (1990) opined that dwarfing gene(s) might become active during the early stages of plant development and could affect numerous other characters either pleiotropically or through linkage. Bushy mutants showed reduction in yield and yield components. Though these mutants may not be useful for direct commercial cultivation because of reduced yield, they may, however, be used in hybridization to transfer some of its useful traits to other high yielding varieties of Bambara groundnut. Jain (1975) suggested that improvement in grain yield in legume crops could be achieved through restructuring of plant type to determinate, erect and compact growth habit.

Various types of leaf mutants for shape like narrow leaved, broad leaved and varied like oval, oblong variants have been recorded were recorded in both the varieties of Bambara groundnut. Such changes were attributed to the chromosomal breakage, disturbed auxin synthesis, disruption of mineral metabolism and accumulation of free amino acids (Gunkel and Sparrow, 1961; Blixt, 1972). Though Bambara groundnut is trifoliate in nature, our studies have reported tetra foliate leaves at 0.3 per cent EMS in variety S-165A. This will increase the biomass production, which could make a positive impact on seed yield.

Earliness is one of those characters of a crop that can be obtained reliably in mutation experiments. Lateness is less desired as a mutant character (Gottschalk and Wolff, 1983). A number of early maturing mutants were isolated at various mutagen treatments in both the varieties of Bambara groundnut. In these mutants, earliness (41 days) was combined with normal seed yield. Such early mutants with altered agronomic characteristics like yield and growth habit were isolated in *Vigna mungo* (Thakur and Sethi, 1993; Kumar *et al.,* 2009). Double seeded mutant, bold seeded mutants serve as a source of useful variation and can be exploited in increasing the number of seeds per pod and seed size leading to increased genetic potential of the yield. Singh, (1996) characterized bold seeded mutants in *Vigna mungo* as due to gene mutations since there were no visible chromosomal changes associated with them.

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