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Induced Mutations in Homozygous and Heterozygous Genotypes of Soybean

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A B S T R A C T

A comparative study of frequency and spectrum of chlorophyll and morphological mutations induced by gamma rays in homozygous genotypes of soybean (*Glycine max* (L.) Merrill) was made. Higher frequency (11.37%) of morphological variants was observed in heterozygous treated material (F_2M_2) than homozygous (7.84%) treated material (M_2). Five types of chlorophyll and ten types of morphological mutants were isolated in M_2 generation. Frequencies of chlorophyll and morphological mutants were higher (0.69 and 1.22% respectively) in treated heterozygous (F_2M_2) material as compared to treated homozygous (0.36 and 1.15% respectively) material (M_2).

Introduction

Soybean (*Glycine max* (L.) Merrill) is one of the most important oilseed-cum leguminous crop gaining importance during recent years. Importance of soybean in Indian Agriculture is obvious because of its high protein (42 %) and oil (20 %) content. In order to induce more variability and utilize useful mutations for efficient plant breeding, comparative assessment of variability generated by hybridization has been reported earlier. The present manuscript, aims to report comparative mutation frequency and spectrum in homozygous and heterozygous genotypes when treated with gamma rays mutagens.

Materials and Methods

Dry seeds of two Soybean (*Glycine max* (L.) Merrill) homozygous cultivars, *viz.*, MAUS-47 and JS-97-52, along with their F_1 hybrid (MAUS-47 x JS-97-52) were treated separately with the gamma rays to 10, 20 and 30 kR dose of gamma rays (CO^{60}) with a dose rate of 2.39 kR per minute at Nuclear and Agriculture Division, B. A. R. C. Trombay, Mumbai-400 085 and the same number of untreated seeds of each varieties served as control.

Ten normal looking plants from each treatments in M_1 were selected randomly to raise M_2 generation. From these, 50 plants per replication were raised in a randomized block design with three replication for each treatment in M_2 generation. Normal cultural practices were carefully screened for chlorophyll and morphological mutations. Mutation frequency was calculated as percentage of M_2 plants.

Results and Discussion

The observed morphological and chlorophyll changes in M_2, F_2M_2 are discussed below (Table 1–5).

M_2, F_2M_2 generation

Chlorophyll mutations

The scoring of chlorophyll mutations in M_2, F_2M_2 generation has been estimated as one of the most dependable indices for evaluation of genetic effects of mutagenic treatments. The mutagenic effect can be measured in both the terms of quantity and quality by studying the spectrum the frequency of chlorophyll mutations.

Gustafsson (1969) has reviewed the various aspects of directed mutagenesis with particular reference to mutagenic specificity and treatment conditions. He postulated that the use of different mutagens under appropriately modifying treatment conditions could alter relative proportions of different types of mutations and enhance the mutations frequency.

The spectrum of chlorophyll mutation induced by mutagenic treatments was found to vary according to the dose and populations. The albina, xantha, viridis, xanthaviridis and chlorina mutations were of common occurrence in all three populations. The different mutagens used, differed significantly from each other for inducing chlorophyll mutations. However ethylmethane sulphonate in all the populations were found most effective in inducing chlorophyll mutations than gamma rays.

All three mutagens *viz*., gamma rays and EMS induced maximum chlorophyll mutations namely albina, xantha, alboviridis, xanthaviridis and chlorophyll. Similar spectrum of chlorophyll mutations was also reported by Amarnath and Prasad (2000) in Tobacco, Geetha and Vaidyanathan (2000), Wakode *et al.*, (2000), Nandanwar *et al.*, (2005), Karthika and Lakshmi (2006), Manjaya (2009), Dhanavel *et al.*, (2012), Magar *et al.*, (2012), Satpute and Fultambkar (2012) and Girija and Dhanavel (2013) in soybean.

The frequency of chlorophyll mutation on M_2, F_2M_2 family basis were found highest in 20 kR gamma rays and 0.6% EMS concentration in all the populations. The similar results were also reported by Dhole (1999), Geetha and Vaidyanathan (2000), Wakode *et al.*, (2000), Bhosale and Hallale (2011) and Makeen *et al.*, (2013).

The induction of chlorophyll mutations may be attributed to changes in structural components of chlorophyll and failure of protoplastid to develop in to plastids with normal size and colour or both (Gustafsson, 1940).

Viable mutations

The prospects of inducing desirable mutants and usefulness in breeding programme have been encouraging since the time of Stadler (1929, 1930).

In the present study, the frequency of viable mutations expressed in M_2, F_2M_2 generation was found to increase in doses of both the mutagens. Frequency and spectrum of viable mutations were high in EMS treatments than gamma rays. This is in contradiction to the earlier reports of Veenakumari (1994) and Paul and Singh (2005) who recorded a greater frequency and wider spectrum of viable mutations in populations treated with gamma rays rather than chemical mutagens.

However, superiority of chemical mutagens over radiations causing functional alterations in genes has been described by Makeen *et al.,* (2013) in urd bean.

The various types of viable mutations with altered plant habit were isolated in M_2 generation and important ones were confirmed in M_3, F_3M_3 generation of all three soybean populations. Study of spectrum of viable mutations showed that numbers of viable mutations were induced for growth habit followed by leaf and then economic mutations.

Among the population JS-97-52 x MAUS-47 was able to express higher frequency and wider spectrum followed by JS-97-52. Similar finding have been reported by Veenakumari (1994), Amarnath and Prasad (2000), Wakode *et al.,* (2000) this suggests that, mutation production and recovery of desirable mutations seems to be mostly governed by genotypic background of the material treated. The mutations confirmed in M_3, F_3M_3 generation are described below.

Small leaf mutant

The mutant isolated for leaf character in which the leaf size was reduced up to one third of control. The leaf shape was found to be elongated and narrow. Similar kinds of mutants were also recorded earlier by Veenakumari (1994) in soybean and Mahadevu (1999) in cowpea.

Broad leaf mutant

These mutants were having broad leaf size. The leaf area of these mutants was observed from 94.48 sq. cm to 113.08 sq. cm in MAUS-47 and from 91.23 to 119.74 sq. cm in JS-97-52 x MAUS-47 Pod bearing of these mutants was medium. Similar type of mutants was also noted by Patil (2006) and Kumar *et al.,* (2009).

Multifoliate mutant

These mutants were isolated in maximum frequency in 0.6% EMS populations. Mahadevu (1999), Maheshwari *et al.,* (2003) and Kumar *et al.,* (2009).

Unbranch mutant

Characteristic feature of these mutant were branchless, trifoliate leaves, medium height and low pod setting. Patil (2006) reported these type of mutant in soybean.

Miniature

These mutants were isolated only in JS-97-52 populations. The height of these mutant was very less. The leaf area was marked by reduced to 10 to 17 sq.cm as against control, 68.50 sq. cm. Similar type of mutant were also reported by Patil (2006) in soybean and Kumar *et al.,* (2009) in urdbean.

High pubescence/Hairy mutant

These mutants were having prominent red and white dense pubescence on stems and pods. The insect attack was found very negligible on these mutants Darange (2004) and Patil (2006) in soybean reported such type of mutants.

Bunchy flower mutant

A very few plants were observed in JS-97-52. The characteristic feature of this mutant is develop flower bunch and not converted to pods. Plant showed luxuriant vegetative growth without reproductive growth. Leaves are quite wider than the normal control populations. Similar type of mutant reported by Nandanwar (1995) in mungbean and Patil (2006) in soybean.

Sterile mutant

These type of mutant was found in the populations of soybean. Sterility was more pronounced at higher doses and with less frequency in JS-97-52. The characteristic feature of these mutants having more branches and very luxuriant vegetative without reproductive growth. Maximum flower on these mutant plants found to be rudimentary nature. The number of flower and anthers in flower were very less. Dhole (1999), Wakode *et al.,* (2000) and Basavaraja (2002) in soybean, Barshile and Apparao (2009) in chickpea reported these types of mutants.

Economic and other mutant

On the basis of earliness, seed colour, size, shape, grain yield plant^{-1} the mutations are classified in to early and late, high and low yielding, bold and small podded and seeded and cream seed vs. Verigated colour seeds, less vs. high branches. Early mutations recorded in all the populations, matured 21 to 34 days earlier than control and were short stature.

Table.1 Frequency of chlorophyll mutations in M_2, F_2M_2 generation of soybean populations

Genotypes	Treatments	No. of seedlings scored	No. of chlorophyll mutants	Frequency of chlorophyll mutations (%)
JS- 97- 52	Control	1521	-	-
	10 kR	1140	8	0.70
	20 kR	1232	11	0.89
	30 kR	1307	9	0.69
	0.4 %	1182	7	0.59
	0.6 %	1042	12	1.15
	0.8 %	1218	10	0.82
MAUS - 47	Control	1422	-	-
	10 kR	1159	5	0.43
	20 kR	1041	11	1.06
	30 kR	1266	14	1.10
	0.4 %	1123	4	0.36
	0.6 %	1294	10	0.77
	0.8 %	1214	12	0.98
JS- 97- 52 x MAUS - 47	Control	1289	-	-
	10 kR	1017	7	0.69
	20 kR	1148	12	1.04
	30 kR	1254	15	1.20
	0.4 %	1178	9	0.76
	0.6 %	1036	10	0.96
	0.8 %	1139	14	1.22

Table.2 Spectrum of chlorophyll mutations in M_2, F_2M_2 generation of soybean populations

Genotypes	**Treatments**	**Chlorophyll mutants**					**Total**
		Albina	**Xantha**	**Chlorina**	**Viridis**	**Xanthviridis**	
JS- 97- 52	Control	-	-	-	-	-	-
	10 kR	-	1	3	2	1	7
	20 kR	3	2	2	3	-	10
	30 kR	2	2	3	4	-	11
	0.4 %	-	1	1	3	-	5
	0.6 %	-	2	3	5	2	12
	0.8 %	1	2	4	6	-	13
MAUS - 47	Control	-	-	-	-	-	-
	10 kR	-	1	2	1	2	6
	20 kR	-	1	4	1	-	6
	30 kR	-	5	3	2	-	10
	0.4 %	-	-	5	3	-	8
	0.6 %	1	3	3	4	-	11
	0.8 %	2	2	3	4	1	12
JS- 97- 52 x MAUS - 47	Control	-	-	-	-	-	-
	10 kR	1	2	1	2	-	4
	20 kR	-	1	6	2	2	11
	30 kR	3	2	3	4	-	12
	0.4 %	-	1	2	3	-	6
	0.6 %	1	2	2	8	1	14
	0.8 %	-	3	4	6	-	12

Table.3 Mutagenic effectiveness and efficiency of mutagens in inducing chlorophyll mutations in soybean populations

Genotypes	Treatments	Mutagenic effectiveness (%)	Mutagenic efficiency (%)
JS- 97- 52	Control	-	-
	10 kR	7	5.23
	20 kR	4.45	4.01
	30 kR	2.3	2.16
	0.4 %	18.43	4.97
	0.6 %	25.83	7.91
	0.8 %	12.81	3.66
MAUS - 47	Control	-	-
	10 kR	4.30	4.44
	20 kR	5.75	8.36
	30 kR	3.43	4.60
	0.4 %	11.25	2.77
	0.6 %	16.04	3.91
	0.8 %	15.31	4.34
JS- 97- 52 x MAUS - 47	Control	-	-
	10 kR	6.9	4.19
	20 kR	5.65	4.85
	30 kR	2.93	2.98
	0.4 %	23.75	5.74
	0.6 %	20.00	6.24
	0.8 %	19.06	4.68

Table.4 Frequency of viable mutations in $M_{2,}$ F_2M_2 generation of soybean populations

Genotypes	Treatments	No. of seedlings scored	No. of viable mutants	Frequency of viable mutations (%)
JS- 97- 52	Control	1521	-	-
	10 kR	1140	10	0.89
	20 kR	1232	19	1.54
	30 kR	1307	26	1.99
	0.4 %	1182	13	1.10
	0.6 %	1042	22	2.11
	0.8 %	1218	21	1.72
MAUS - 47	Control	1422	-	-
	10 kR	1159	12	1.04
	20 kR	1041	18	1.73
	30 kR	1266	16	1.26
	0.4 %	1123	15	1.33
	0.6 %	1294	32	2.47
	0.8 %	1214	26	2.14
JS- 97- 52 x MAUS - 47	Control	1289	-	-
	10 kR	1017	14	1.38
	20 kR	1148	16	1.39
	30 kR	1254	28	2.23
	0.4 %	1178	21	1.78
	0.6 %	1036	30	2.90
	0.8 %	1139	29	2.55

Table.5 Spectrum of viable mutations in M_2, F_2M_2 generation of soybean populations

Genotypes	Treatments	Leaf mutant			Growth habit mutant				Other mutants		
		Narrow leaves	Broad leaf	Multifoliate leaf	Dwarf bushy	Tall erect	Unbranch	miniature	Changed flower colour	High pubesence	Bunchy flower
JS – 97 - 52	Control	0	0	0	0	0	0	0	0	0	0
	10 kR	-	1	-	2	3	-	2	2	-	1
	20 kR	2	-	1	1	-	-	-	-	-	-
	30 kR	9	-	-	2	1	-	1	-	-	-
	0.4 %	2	-	1	9	4	-	-	2	2	-
	0.6 %	8	-	2	6	1	-	1	4	-	-
	0.8 %	4	-	-	5	-	-	-	-	-	2
MAUS - 47	Control	0	0	0	0	0	0	0	0	0	0
	10 kR	1	1	-	2	1	8	-	-	-	-
	20 kR	4	-	-	5	3	7	-	-	-	-
	30 kR	3	3	1	-	-	2	-	-	-	-
	0.4 %	2	-	1	2	3	12	-	-	1	3
	0.6 %	7	1	1	14	1	10	-	-	-	-
	0.8 %	-	2	4	1	3	-	-	-	2	2
JS- 97- 52 x MAUS - 47	Control	0	0	0	0	0	0	0	0	0	0
	10 kR	2	-	1	3	1	-	-	-	-	1
	20 kR	3	-	-	1	1	4	1	-	3	-
	30 kR	2	2	1	-	3	1	-	-	2	4
	0.4 %	3	12	-	9	7	-	-	-	-	-
	0.6 %	-	2	-	3	2	8	-	-	-	-
	0.8 %	1	-	6	7	-	13	-	-	-	-

Table.5 Contd…………

Genotypes	Treatments	Other mutants				Economic mutant					Total mutants
		sterile	Small podded & seeded	Deformed pod	Verigated seed colour	Early maturing	Late maturing	High branching	Big podded & bold seeded	Non shattering	
JS- 97- 52	Control	0	0	0	0	0	0		0	0	0
	10 kR	-	5	-	-	3	5	3	2	3	32
	20 kR	1	12	-	-	1	-	-	4	9	31
	30 kR	1	2	-	1	-	-	-	-	7	24
	0.4 %	2	-	2	-	2	6	4	2	10	48
	0.6 %	-	4	1	-	4	3	8	4	8	54
	0.8 %	3	10	-	1	2	3	5	3	14	52
MAUS - 47	Control	0	0	0	0	0	0	0	0	0	0
	10 kR	1	-	1	-	7	3	2	3	-	30
	20 kR	5	2	-	-	4	5	1	6	3	45
	30 kR	1	-	-	-	-	2	3	9	4	28
	0.4 %	-	1	-	-	2	2	-	1	1	31
	0.6 %	4	-	1	-	-	-	3	7	5	54
	0.8 %	2	1	-	-	2	-	-	-	3	22
JS- 97- 52 x MAUS - 47	Control	0	0	0	0	0	0	0	0	0	0
	10 kR	1	6	-	-	3	2	1	3	2	26
	20 kR	5	3	2	1	3	6	6	10	7	53
	30 kR	1	1	-	1	7	2	3	5	7	42
	0.4 %	3	3	1	-	13	1	-	-	3	55
	0.6 %	7	9	4	-	2	10	7	7	7	66
	0.8 %	4	1	2	1	5	-	10	9	10	69

Late maturing recorded 20 to 25 days delayed maturity as compared to control. High yielding mutants were medium tall, healthy, with increase pod bearing branches, pod number and grain yield. High yielder recorded double grain yield as compared to control plant. Some of these mutants bred true in M_3, M_3F_3 generation. Some other types of mutants like long and small podded, deformed pod, high branching, non-shattering of pod, small and bold seeded, Verigated seed shape and colour isolated in all the populations in M_2, F_2M_2 generation, Makeen *et al.,* (2013) in urd bean, and Ahire *et al.,* (2005) and Patil (2006) reported this type of mutant in soybean.

Mutagenic effectiveness and efficiency

Mutagenic effectiveness is a measure of the frequency of mutations induced by a unit dose of mutagen while mutagenic efficiency is the proportion of mutations in relation to other associated undesirable changes such as lethality, and sterility (Konzak *et al*., 1965) induced by a mutagen.

The usefulness of any mutagen in plant breeding depends not only on its mutagenic effectiveness but also on its mutagenic efficiency. Efficient mutagenesis is the production of desirable changes with minimum undesirable changes.

The efficiency of a mutagenic agent is a complex question as it not only depends on the agent with the material and on its applicability to the biological system but also on the degree to which physiological damage, chromosomal aberrations and sterility is induced in addition to mutations.

In the present investigation, in general lower doses of both the mutagens were effective in producing chlorophyll mutations indicating that increase in chlorophyll mutations frequency was not proportional to the increase in dose. Among the two mutagens tried, EMS was highly effective compared to gamma rays. This is in conformity with the reports of Konzak *et al.,* (1965) in barley, Veenakumari (1994) in soybean.

The estimates of mutagenic efficiency in general indicated that the efficiency of EMS was more compared to gamma rays in JS-97-52 x MAUS-47 and JS-97-52. This is in accordance with the finding of Konzak *et al.,* (1965) in lathyrus, Amarnath and Prasad (2000) in tobacco, Patil (2006) in soybean. Contrary to this, gamma rays showed more efficiency in MAUS-47, which is similar to the finding of Nandanwar and Khamankar (1996) and Gautam *et al.,* (1998) who reported that the efficiency of same mutagen varied in different genotypes of urd bean, Pavadai *et al.,* (2010), Satpute and Fultambkar (2012) in soybean.

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