academicJournals

Vol. 8(47), pp. 5958-5961, 5 December, 2013
DOI: 10.5897/AJAR07.038
ISSN 1991-637X ©2013 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Study of chlorophyll and macromutations induced by gamma rays and sodium azide in urd bean (*Vigna mungo* L. Hepper).

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Accepted 24 January, 2011

The mutagenic effect of gamma rays (5, 10, 20, 30, 40, 50 and 60 kR) and sodium azide (1, 2, 3, 4, 5, 6 and 7 mM) alone or in combination (10 kR + 2 mM, 20 kR + 2 mM, 30 kR + 2 mM and 40 kR + 2 mM) on frequency and spectrum of chlorophyll and micromutations in cultivar, T9 of urd bean have been observed. The spectrum of chlorophyll mutations consisted of albina, chlorina, viresence, viridis and xentha. Out of these chlorophyll mutations, xantha type was predominant in both mutagenic treatments. Conclusively, the combination treatments have yielded the higher frequency, and various doses of mutagenic agents have independent response towards macromutations in bean cv-T9. These studies were broadly aimed at understanding the process of mutation, testing the efficacy of various mutagens, identifying optimum dose and best method of treatment; isolation of mutants of basic and applied value; elucidating the biological effects of gamma rays and sodium azide.

Key words: Gamma rays, sodium azide (SA), chlorophyll, macro mutations, urd bean.

INTRODUCTION

Mutation breeding is relatively a quicker method for improvements of crops. It has been observed that induced mutations can increase yield as well as other quantitative traits in plants. Since the discovery of radiation and chemical mutagens, a large amount of work has been done on study of radio sensitivity of crop plants with ionizing radiations and chemical mutagens (Caldecott, 1955). Many physical and chemical mutagens have been used for induction of useful mutants in a number of crops. A completely different mood of action has been indicated for sodium azide, compared to gamma rays, (Kleinhofs et al., 1975; Kanzak et al., 1965). The two mutagens gamma rays and sodium azide (SA) belong to two different categories. Therefore, investigations were undertaken to study the comparative mutagenicity of these mutagens under similar treatment conditions. Chlorophyll mutations although not useful for plant breeding purpose, may be used to assess the efficiency and effectiveness of mutagens in order to select suitable mutagen at appropriate concentration so as to use them in applied mutagenesis programme. In the present study, the effect of gamma rays and sodium

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Abbreviations: RBD, Randomized block design, **SA,** sodium azide.

the frequency and spectrum of chlorophyll mutations and viable macro mutations in M_2 generation in urd bean. azide employed singly or in combinations was studied on

MATERIALS AND METHODS

Seeds of Urd bean (*Vigna mungo*) cultivar T9 were used for inducing mutation by gamma rays, SA and their combinations. For each treatment, 300 uniform and dry seeds were used. Seeds were irradiated by exposing them to 5, 10, 20, 30, 40, 50, and 60 kR (IR = 2.58 x 10^{-4} C/kg) of gamma rays from ^{60}Co source in gamma cell at NBRI – Lucknow, India.

For treatment with chemical mutagen SA, seeds were presoaked in distilled water for 8 h and then treated with buffered solution (pH 7.0) of SA at 5, 10, 20, 30, 40, 50, 60, and 70 mM concentrations for 6 h after which the seeds were thoroughly washed in running tap water. For combination treatment that is, gamma irradiation followed by SA, only one concentration of SA (20 mM) was used in combination with 10, 20, 30, and 40 kR gamma rays.

After completion of the treatment with gamma rays, SA and their combinations along with their respective control seeds were sown immediately to raise the M_1 generation in a randomized block design (RBD) with three replications. Different biological parameters like germination, survival of plant, pollen sterility and ovule sterility were recorded, and expression as a percentage of controlling randomly marked plants in each treatment in M_1 generation were harvested separately.

For raising of M_2 generations, the seeds of randomly selected plants of M_1 generation were space planted in the field in three replications. M_2 generation was screened for lethal chlorophyll mutuations during the first four weeks, after germination. Whereas, viable chlorophyll and macromutants were scored throughout the crop duration. The population was screened for chlorophyll and macromutations according to the procedure given by Gustafesson (1947) with suitable modifications. Mutations frequency was calculated by the following methods given by Gaul (1957):

$$\text{Mutation frequency} = \frac{\text{No. of mutated progenies}}{\text{Total No. of progenies}} \times 100 \quad (1)$$

$$\text{Mutation frequency} = \frac{\text{No. of mutated Plants}}{\text{Total No. of Plants}} \times 100 \quad (2)$$

RESULTS AND DISCUSSION

It is evident from the data (Table 1) that higher doses/concentrations of mutations were more effective in inducing greater frequency of chlorophyll mutations in cv. T9. The chlorophyll mutation frequency on the M_2 plant basis increased with the increase in the doses of gamma rays, sodium azide and their combination. The increased cholorophyll mutation frequency at higher doses may be attributed to the chromosomal aberrations or saturation in the mutational events which may result in the elimination of mutant cells during growth (Brock, 1965). Combined effect of gamma rays and SA were most effective in this respect. The spectrum of chlorophyll mutants induced by gamma rays SA and their combination included albrina, chlorina, virescence, virids and xantha. Among these five chlorophyll mutants, xantha type was predominant in both mutagens, suggesting that genes for Xanthophyll development are readily available for mutagenic action. Cholrina type was found in the least frequency. Similar observations were made by Reddy and Anandurai (1991) in lentil.

The highest frequency of chlorophyll mutations (2.95%) was induced by 40 kR gamma rays followed by 60, 50, and 20 kR as 2.66, 2.64 and 2.30% respectively. Whereas, incase of Sodium azide highest frequency of chlorophyll mutations (3.36%) was found at 40 mM, followed by 2.55, 2.52, and 2.38% at 50, 70, and 60 mM respectively. Among combination treatment highest frequency of chlorophyll mutations (3.80%) was found at (40 kR + 20 mM). Interestingly, lower chlorophyll mutations were found at lower doses gamma ray, sodium azide and their combination. Similar observations were made by Mehraj-ud-din et al. (1999) in Mung bean.

The spectrum of macro mutations showed in Table 2 was higher by sodium azide than gamma rays. But higher frequency of macromutations was found by the combination gamma rays and sodium azide. In the case of gamma rays, the mutation spectrum was much wider (seven types) at 40 kR followed by 50 kR (Six type). Whereas, in case of sodium azide, the mutation spectrum was much wider (seven types) at 40 mM. On the other hand, among combination treatments, the mutation spectrum was wider (seven types) at 10 kR+ 20 mM and 20 kR + 20 mM followed by 30 kR + 20 mM (six types).

The above findings are in accordance to the observations of several other workers, Singh et al. (1999) in Urd bean, Thakur and Sethi (1995) in barley. Several macro mutants leading to aberrant plant type (tall, erect and bushy), plant habit (ten driller plant with slender stem), leaf (tetra foliate and pent foliate in contrast to normal trifoliate), inflorescence (multiraceomose - plant bearing numerous racemes), disease resistant (as cv.T9 is yellow mosaic susceptible), and early maturing could occur in cv.T9 at a fair frequency in M_2.

Several other workers have reported the occurrence of chlorophyll mutations after mutugenic treatment in pigeon pea (Venkateswarlu et al., 1978) and in mung bean (Singh and Yadav, 1991). Chlorophyll mutation rate is a critical parameter to determine the effectiveness and efficiency of treatment of different mutagens. An interesting observation is that genes near the Centro mere were more prone to mutagenic treatment than those located farther away. Further, chlorophyll mutants were frequent in SA treatment but were rare in treatments with physical mutagens (Pal et al., 1998).

At this time, this result was attributed to differences in chemical composition of the chromosomes near Centro, mere making them more sensitive to chemical mutagens. While it may indeed be the case, other explanations are possible. For example, genes near the centromere are less likely to be involved in recombination and hence mutations in those genes are less likely to be eliminated through selection. Mutations in these chlorophyll genes

Table 1. Frequency and spectrum of chlorophyll mutations in M_2 generation of Black gram (*vigna mungo* L.) cv. T9.

Treatment	Dose	No. of M_1 plant progenies	No. of plant progenies segregating in M_2	% Mutated plant progenies	No. of plant in M_2	Relative percentage of different chlorophyll mutants					Total frequency (%)
						Albina	*Chlorina*	*Viresence*	*Viridis*	*Xantha*	
Gamma rays	5	192	18	34.56	3954	17.0	-	13.0	9.52	35.04	1.88
	10	185	25	46.25	3748	-	9.45	20.0	17.31	20.0	1.76
	20	174	35	60.9	3603	11.35	16.13	18.30	18.18	19.00	2.3035
	30	118	58	68.44	3200	18.14	19.11	-	17.34	19.0	2.2987
	40	105	85	89.25	2930	15.13	22.22	13.0	15.17	21.15	2.95
	50	96	90	86.4	2700	17.35	-	19.0	15.13	20.0	2.647
	60	80	72	57.6	2100	15.23	9.30	-	15.50	16.00	2.66
Sodium azide (mM)	1	200	17	34.0	3700	-	20.15	12	14.24	23.00	1.87
	2	190	19	36.1	3624	12.12	13.27	-	11.16	20.14	1.56
	3	174	25	43.5	3400	12.12	-	16.37	14.20	16.00	1.72
	4	104	41	42.64	2200	18.12	11.00	-	9.13	20.75	3.36
	5	96	47	45.12	1750	-	-	13.17	16.21	15.31	2.55
	6	90	56	50.4	1800	17.80	-	13.21	12.0	-	2.38
	7	70	50	35.0	1600	9.15	10.10	11.14		10.13	2.520
Combination (gamma rays + SA)	10 kR + 2 mM	125	35	43.75	2800	15.24	20.21	17.35	-	21.13	2.6403
	20 kR + 2 mM	112	48	53.76	3200	14.14	18.21	13.00	17.13	-	2.55
	30 kR + 2 mM	96	40	38.4	2500	1700	-	-	19.23	19.00	2.20
	40 kR + 2 mM	56	48	26.88	1200	11.23	-	12.00	10.15	12.23	3.80

Table 2. Spectrum and frequency of micro mutations in M_2 plants in Urban Cultivar T9 induced by physical and chemical mutagens.

Treatment	Dose	No. of plant in M_2	Tendriller	Tall and erect	Bushy	Trifoilate	Pentafoilate	Disease resistant	Early maturing	Total frequency (%)
Gamma rays (kR)	5	3954	11	4	7	6	-	15	2	1.13
	10	3784	-	8	8	8	-	17		1.08
	20	3603	10	9	4	-	-	14	2	1.08
	30	3200	9	-	8	2	4	-	10	1.03
	40	2930	14	11	8	7	4	14	7	2.24
	50	2700	10	14	7	6	-	13	4	2.00
	60	2100	9	11	-	13	-	11	-	209
Sodium azide (mM)	1	3700	-	8	8	4	3	11	5	1.05

Table 2. Contd.

	2	3624	7	5	-	8	2	13	2	1.02
	3	3400	17	4	7	-	-	7	8	1.26
	4	2200	14	10	10	-	-	14	8	2.54
	5	1750	-	-	13	7	-	13	10	2.45
	6	1800	8	13	-	-	-	11	7	3.16
	7	1600	8	12	10	3	-	-	-	2.06
Combination treatment (Gamma rays + SA)	10 kR + 2 mM	2800	8	10	10	4	8	18	4	2.21
	20 kR + 2 mM	3200	14	10	13	2	7	15	9	2.18
	30 kR + 2 mM	2500	15	10	8	-	9	12	8	2.48
	40 kR + 2 mM	1200	-	4	8	-	-	13	7	2.66

may induce chlorophyll mutations. SA acts through the inhibition of catalase and peroxidase (Kleinhof, 1978). As a respiratory inhibitor Sodium azide may inhabit energy supply system resulting in the inhibition of mitosis, which can be associated with seedling growth depression (Nilan et al., 1973). Therefore, SA causes drastic chlorophyll mutations than those induced by radiations, probably because of their apparently less drastic effect on chromosome (Nilan, 1972).

The genetic differences caused by two mutagens inducing spectrum and frequency of macromutants have been observed by Nerkar and Mote (1978) in Bengalgram, Sharma and Sharma (1981) in lentil and Rao and Reddy (1984) in Pigeon pea reported mutations for plant type, branching pattern, leaf morphology, penduncle length, pod length, seed color and boldness, etc. in mung and urd beans by radiations and chemical mutagens employed alone or in combination. This possible cause of these macro mutations may be chromosomal aberrations, small deficiencies or duplications and most probably gene mutations.

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