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POST-IRRADIATION AGEING EFFECT ON MORPHOLOGICAL CHARACTERS OF *CROTALARIA SALTIANA*

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Abstract

Post-irradiation ageing effect on seed germinability and different morphological characters of *Crotalaria saltiana* were studied in the year of 2006. Germination percentage of *Crotalaria saltiana* seeds treated with different doses of gamma rays, aged for 32 years in moisture free condition showed remarkable variation. Plants grown from the treated seeds of all the doses showed diversity among themselves regarding their morphological characters. Such as plant height was found to reduce in lower doses and this character showed no such consistent result. Leaf irregularities such as, invaginated margin, inverted margin with blunt apex, leaf with bifurcated apex, small size and dented margin, etc. were recorded.

Key words: Post-irradiation ageing effect, Morphological characters, *Crotalaria saltiana*.

Introduction

The species *Crotalaria saltiana* belongs to the sub-family Papilionaceae of the family Leguminosae and it has been growing naturally under the prevailing environmental condition of Bangladesh. This species is useful sometimes for nitrogen fixation by their root nodule.

Ageing is the universal physiological phenomenon occurring in living organisms. Usually it starts with a faster rate under stresses of unfavorable condition and normally proceeds at a slower pace as programmed by the genetic information specific to the species under adapted condition (Strehler 1962). Ageing in stored seeds is a well known fact (Crocker and Barton 1983) and it has become a problem in modern agricultural practices (Nutile 1974). The ageing effect in plant seeds induced recessive gene mutations of leaf and flower characters as it was reported by Stubbe (1995), who found that *Antirrhinum* plants grown from 10 years old seeds had 7 times as many mutations as did plants grown from 6 years old seeds. Ageing of *Antirrhinum* pollen increased the mutation rate. Pollen aged for 10 weeks before pollination produced more than twice as many mutations as did pollen aged for 4 weeks before pollination. The ageing effect is more rapid in the pollen than it is in the plant seeds.

Seed longevity is known to vary greatly among species and within species. Haferkamp *et al*. (1993) showed that 96% of barley seeds germinated after 32 years storage in an unsealed condition at air temperature. Robertson *et al*. (1999) found that germination of barley dropped to 46.20% of the initial germination after 21 years in a day, unheated room in Colorado, indicating a much shorter longevity of barley seeds. Storage conditions, particularly temperature and seed conditions are the main factors influencing seed longevity.

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Abnormal seedlings raised from aged seeds have been observed in various plant species. Necrotic abnormalities in roots and shoots and non-survival of some seedlings were reported in aged seeds of *Pisum sativum* (Murata *et al*. 1996). The delay of the first mitosis in root tip cells induced by artificial seed ageing is associated with slow activation of cell division following the start of first mitosis. Therefore, any delay in the first mitosis caused by seed ageing should be taken in to consideration in scoring chromosomal aberrations in mitosis (Murata *et al*. 1991). Emery *et al.* (1990) made the opinion that irradiation breeding may be possible alternative and supplement to hybridization as a source of creating genetic variability and proper utilization of the same. However, in order to carry out mutation breeding experiment in any biological material, the knowledge of its radio sensitivity is of primary importance. The effect of irradiation on *C. saltiana* has been reported several times. But information regarding effect of natural ageing on irradiated plant materials is not available. Particularly this type of work on *C. saltiana* is scanty. The present study was aimed to see the morphological changes in plants raised from thirty two years (irradiated) naturally aged seeds of *C. saltiana.*

Materials and Methods

Thirty two years old irradiated seeds (seeds were treated in the year of 1974) of *C. saltiana* were used as plant material. Dry seeds of *C. saltiana* were irradiated with gamma rays from 5000 ci Co^{60} source at the rate of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Kr from the Atomic Energy Center, Dhaka. These irradiated seeds from preserved condition were supplied by Professor Sultanul Alam Cytogenetics Laboratory, Department of Botany, Rajshahi University, Rajshahi for the present study. The experiment was conducted in the year of 2006.

For germination performance seeds from each of the doses were placed on moist filter papers in Petri dishes in laboratory. It was also done in three replications using earthen pots containing well manured soil for germination test. After seven days of setting the experiment in laboratory and after twelve days of sowing the seeds in pots germination data were taken. Data on different morphological characters were also recorded from the plants raised in all the doses from the treated naturally aged seeds of grown in pots. Observation was made for any qualitative changes in different morphological parts. The findings obtained from the experiment were analyzed statistically.

Results and Discussion

Studies on germination percentage of *C. saltiana* seeds treated with different doses of gamma rays and aged of 32 years in moisture free desicator in the laboratory and pot showed remarkable variation. In Petri dishes the highest germination percentage (61.00%) was observed due to 30 Kr and the lowest (27.66%) was found due to 50 Kr which were significantly different from that of all other treatments. In case of pot the highest germination percentage (60.00%) was observed due to 40 Kr which was significantly different from that of all other treatments. On the other hand, the lowest (30.00%) was found due to 70 Kr and that was significantly different from that of all other treatments except 20 and 100 Kr (Table 1). Post irradiation ageing affected the seed germinability but it may be said that the effect could be more lethal when ageing period is taken into consideration. The results obtained with aged seeds of *C. saltiana* demonstrated the notable deletorious effect of age. Akhter *et al.* (1992) reported that germination percentage of different year's old wheat and barley seeds were decreased gradually with an increase of the storage time.

Table 1. Post-irradiation ageing effects on germination percentage and plant height at different days after sowing (DAS) of *Crotalaria saltiana*.

Treatments	Germination % in laboratory	Germination % in pot	Plant height (cm)				
			20 DAS	40 DAS	60 DAS	80 DAS	At maturity
10 Kr	50.00b	35.00d	2.80d	4.50f	20.00d	50.30f	79.20g
20 Kr	51.00b	31.00e	6.50b	13.20bcd	25.30c	58.40d	94.80e
30 Kr	61.00a	34.23d	6.00b	17.50a	35.00a	70.00a	114.00a
40 Kr	40.00d	60.00a	7.50a	14.00bc	33.00b	60.30c	93.50e
50 Kr	27.66f	51.00b	4.50c	15.00b	29.10b	62.10b	101.50c
60 Kr	41.00d	40.00c	6.00b	13.50bcd	29.50b	60.20c	98.50d
70 Kr	40.00d	30.00e	4.50c	12.50cd	30.00b	62.20b	109.50b
80 Kr	36.48e	49.00b	2.50d	7.50e	25.50c	56.10e	91.10f
90 Kr	44.00c	39.00c	2.20d	12.00d	-	-	-
100 Kr	40.00d	31.00e	2.00d	-	-	-	-
CV (%)	6.25	8.26	6.48	9.08	3.61	1.63	1.20

Means followed by the same letter(s) do not statistically differ at 5% level tested by DMRT. No values for plant height against 90 and 100 Kr indicate death of the plants at seedling stage.

Khan (1981) recorded 96-100% seed germination (irradiated woth 10-100Kr gamma rays) of two *Clotaria* Species viz. *C. juncea* and *C. sericea.* The present findings indicated a remarkable variation in germination percentage due to ageing in irradiated seeds of *C. saltiana.* However, no such effects of doses (low and high) was found on increasing or decreasing of seed germination. Rather, the germination percentage due to a dose of gamma ray was low. The findings suggested indeed that the viability of seeds could be protected by keeping them in moisture free desicator in laboratory condition for long time.

The plant height at different days after sowing (DAS) is shown in Table 1. At 20 DAS the maximum plant height (7.50 cm) was observed in 40 Kr which was significantly different from that of all other treatments. Whereas the minimum plant height (2.00 cm) was found in 100 Kr and that was statistically identical with that of 10, 80 and 90 Kr. At 40, 60 and 80 DAS and at maturity the maximum plant heights (17.50, 35.00, 70.00 and 114.00 cm) were observed in 30 Kr and the minimum (4.50, 20.00, 50.30 and 79.20 cm) were found in 10 Kr, respectively which were significantly different from that of all other treatments (Table 1). The maximum leaf length (4.21 cm) was observed due to 10 Kr which was statistically identical with that of 20, 50 and 80 Kr (Table 2) and the minimum (2.70 cm) was observed due to 60 Kr and that was significantly different from that of all other treatments. Leaf breadth and petiole length were statistically nonsignificant.

The highest inflorescence length (41.57 cm) was observed in 30 Kr and the lowest (28.87cm) was due to 70 Kr which were significantly different from that of all other treatments (Table 2). Almost in all the doses inflorescence was not found to be healthy. The maximum value for fruit length (4.36cm) was observed in case of 10 Kr, which was statistically identical with that of 20, 30, 40, 60 and 70 Kr (Table 2) and the minimum (3.64cm) was in 80 Kr and that was statistically identical with that of 50 Kr. Table 2 shows that highest number of seeds/fruit (47.10) was found due to 20 Kr and this value was statistically identical with that of 10, 30 and 40 Kr. On the other hand, the lowest (17.00) was found in case of 50 Kr and it was significantly different from that of all other treatments except 80 Kr. The maximum value for 100 seed weight (0.730 g) was found due to 80 Kr and it was statistically identical with that of all other treatments except 20 and 30 Kr (Table 2) and the minimum (0.560 g) was found due to 30 Kr which was statistically identical with that of all other treatments except 80 Kr.

Table 2. Post-irradiation ageing effects on different morphological characters of *Crotalaria saltiana*.

Treatments	Leaf length (cm)	Leaf breadth (cm)	Petiole length (cm)	Inflore-scence length (cm)	Fruit length (cm)	No. of seeds/fruit	100 seed wt. (g)
10 Kr	4.21a	2.26a	0.28a	35.07c	4.36a	44.20a	0.614ab
20 Kr	4.10a	2.33a	0.24a	38.07b	4.16a	47.10a	0.563b
30 Kr	3.74b	2.19a	0.25a	41.57a	4.31a	40.60a	0.560b
40 Kr	3.84b	2.21a	0.27a	33.63b	4.24a	38.30ab	0.510ab
50 Kr	4.11a	2.41a	0.28a	38.53b	3.72b	17.00e	0.665ab
60 Kr	2.70c	2.04a	0.23a	30.86d	4.19a	26.80cd	0.660ab
70 Kr	3.92b	2.24a	0.29a	28.87e	4.10a	31.40bc	0.622ab
80 Kr	4.11a	2.35a	0.28a	34.10c	3.64b	19.10de	0.730a
CV (%)	12.35	15.36	19.36	3.89	16.42	19.29	17.15

Means followed by the same letter(s) do not differ at 5% level tested by DMRT. No values against 90 and 100 Kr indicate death at seedling stage.

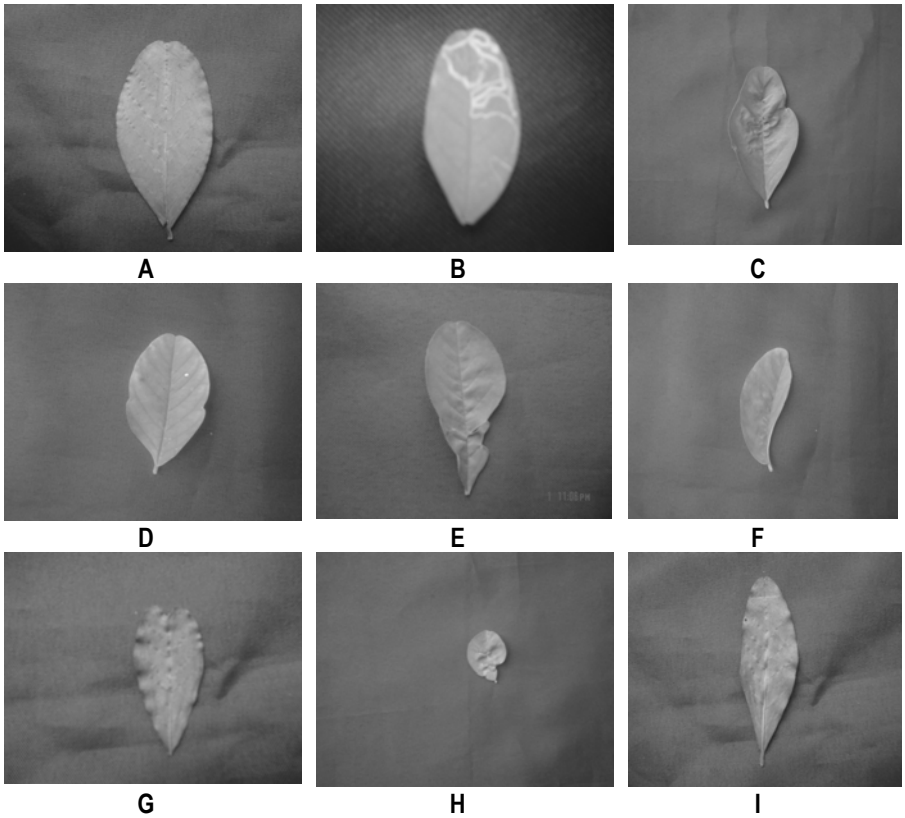

Plate 1. Photographs showing different types of leaf abnormalities induced by gamma rays and ageing along with normal in *Crotalaria saltiana*. A. Normal leaf, B. Curved apex with chlorophyll mosaic, C. Invaginated margin, D. Invaginated margin with blunt apex, E. Crinkled leaf with dented margin, F. Narrow leaf with curved margin, G. Leaf with bifurcated apex, H. Small size and dented margin and I. Narrow leaf with invaginated apex.

In *C. saltiana* no morphological abnormalities of leaf were observed due to the 10 Kr but the plants raised from the seeds treated with higher doses showed different types of leaf abnormalities. The abnormalities were curved apex with chlorophyll mosaic, invaginated margin, inverted margin with blunt apex, crinkled leaf with dinted margin, narrow leaf with curved margin, leaf with bifurcated apex, small size and dented margin, narrow leaf with invaginated apex etc. In addition to this leaf abnormalities one or two plants from certain doses showed bifurcation or trifurcation stem at seedling stage (Figures 1-9).

In the present study, plants due to different doses under study showed diversity among themselves regarding their morphology. Post irradiation ageing affected the plant height and it was found to decrease at the high dose. It might be due to both gamma ray and ageing effect. A gradual decrease in plant height was observed from low to high doses of gamma radiation (Islam *et al*. 1994). Khan and Alam (1982) detected different types of morphological abnormalities, reduced seedling height and poor germination rate in *C. juncea* and *C. sericea* treated with different doses of gamma rays. Srivastava (1983) observed a reduction of shoot length and plant height of wheat plants after gamma rays treatment.

Khan (1981) reported variations among ten doses of four *C.* species regarding plant height, leaf length and breadth, petiole length, stomata length, pollen grain diameter, fruit length, fruit diameter, number of seeds/fruit, seed length and breadth and 100 seed weight. Islam *et al*. (1994) also reported morphological abnormalities of *C. juncea* due to treatment with gamma rays and temperature for 12 to 36 hours. The findings regarding abnormalities in leaves of *C. saltiana* was found to be supported by the findings of Islam *et al*. (1994) and Khan and Alam (1982).

Present findings indicate significant effect of radio-sensitivity even after a long time of natural ageing. Thus, it may be concluded that in breeding programme the irradiated seeds may be used as a source of creating genetic variability, if they are stored artificially or naturally for a long time.

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