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RADIOSENSITIVITY OF *ALBIZIA PROCERA* TO ACUTE AND FRACTIONATED GAMMA IRRADIATION AT TWO MOISTURE LEVELS

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AHLAWAT, S.P. & KAPOOR, M.L. 1997. Radiosensitivity of *Albizia procera* to acute and fractionated gamma irradiation at two moisture levels. Radiosensitivity of *Albizia procera* was calculated by exposing the seeds to acute and fractionated doses (0, 200, 400, 800, 1200 and 1600 Gy) of gamma rays at two moisture levels (6.5% and 48%). $ED_{50/20}$ (effective dose) was estimated for seed germination, survival, growth and biomass of seedlings. Chromosomal aberrations were also scored at mitotic anaphase. The number of abnormal cells increased with the increasing doses of gamma rays. Presoaked seeds were more sensitive to gamma rays than those air-dried. $ED_{50/20}$ doses of dry seeds under acute irradiation were 540 Gy and 770 Gy for shoot and root lengths respectively. Corresponding values for presoaked seeds were 135 Gy and 140 Gy respectively. The fractionated mode of irradiation showed comparatively more reductions in values of different parameters than acute irradiation for both dry and presoaked seeds of *Albizia procera.* This information could be of significance in further planning mutation breeding strategies and changing the spectrum of induced mutations by altering the mode of irradiation and moisture level of seeds.

Keywords: *Albizia procera* - acute - fractionated - gamma rays - radiosensitivity - $ED_{50/20}$

AHLAWAT, S.P. & KAPOOR, M.L. 1997. Radiosensitiviti *Albizia procera* kepada sinar gamma akut dan pecahan pada dua aras lembapan. Radiosensitivi *Albizia procera* dikira dengan mendedahkan biji benih kepada dos akut dan pecahan (0, 200, 400, 800, 1200 and 1600 Gy) sinar gamma pada dua aras lembapan (6.5% dan 48%). $ED_{50/20}$ (dos berkesan) ditaksirkan bagi percambahan biji benih, kemandirian, pertumbuhan dan biojisim anak benih. Aberasi kromosom juga dicatatkan pada peringkat anafasa mitosis. Bilangan sel abnormal bertambah dengan bertambah-nya dos sinar gamma. Biji benih yang diprarendam lebih sensitif kepada sinar gamma daripada biji benih yang dikering udara. Dos $ED_{50/20}$ anak benih kering di bawah sinar akut ialah 540 Gy dengan 770 Gy bagi panjang pucuk dan panjang akar. Nilai sepadan bagi biji benih yang diprarendam masing-masing ialah 135 Gy dan 140 Gy. Sinaran dengan mod pecahan menunjukkan lebih pengurangan dalam nilai perameter berlainan berbanding dengan sinaran akut bagi biji benih *Albizia procera* yang kering dan yang diprarendam. Maklumat berikut boleh mempunyai makna dalam cadangan strategi pembiakan mutasi dan mengubah spektrum mutasi teraruh dengan mengubah mod sinaran dan aras lembapan bagi biji benih.

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Introduction

Mutation breeding has played a significant role in the improvement of many agricultural and horticultural crops (Micke 1984). Gustafsson (1960) and Sparrow *et al.* (1968) have suggested the use of mutation breeding in forestry for tree improvement. However, its potential in tree breeding for producing superior genotypes has not been fully explored as most of the tree species are cross-pollinated, and have a long life cycle. It is possible to improve the tree species in a short time if mutation breeding is used in conjunction with conventional methods and tissue culture techniques.

Albizia procera, a leguminous tree, fixes the atmospheric nitrogen and improves soil fertility. In addition, it is used as food, fodder, fuel and timber. Being a multipurpose tree it occupies a prominent place in social and agroforestry programmes. It is also planted as nurse tree to provide shade in coffee and tea plantations. Therefore, improvement in qualitative and quantitative traits of *A. procera* assumes a great significance.

Information on the sensitivity of species and effectiveness of gamma doses for causing maximum mutations is a pre-requisite for successful mutation breeding. In this paper results are reported on the radiosensitivity of *Albizia procera* and $ED_{50/20}$ values of gamma rays for various traits under different modifying factors.

Material and methods

Air-dry seeds and seeds presoaked in water for 8 h (moisture 6.5% and 48.0%, respectively) were irradiated with acute and fractionated doses of gamma rays. The doses given were 0, 200, 400, 800, 1200 and 1600 Gy with a dose rate of 0.37 Gy/second, from a Co-60 source at the Radioisotope Laboratory of the Forest Research Institute, Dehra Dun. Each treatment had 100 seeds with four replications, and additional replication was kept for cytological studies. Fractionated doses were given in two spells with a gap of 48 h. Irradiated seeds were kept on moist germination paper in Petri dishes in a seed germinator maintained at 30 ± 2 °C temperature and 80% humidity.

From the daily germination count, germination value (GV) was calculated according to Czabator (1962) and germination energy index (GEI) according to the revised formula of Groese and Zimmer (1958). Two weeks after sowing the root and shoot lengths and cotyledon length and width were measured. Fresh and dry weights of seedlings were also recorded at the end of the experiment. The reduction percentage values with respect to the control were calculated to obtain (effective dose) $ED_{50/20}$ values. The root-tips were fixed in freshly prepared solution containing 1:3 acetic-alcohol for 48 h and then transferred to 70% alcohol for storing. Root-tip squashes were prepared in 1% acetocarmine. Chromosomal aberrations at anaphase were scored and percentage of abnormal cells calculated.

Results and discussion

The radiosensitivity of *Albizia procera* under two moisture levels gave a differential response to acute and fractionated doses of gamma rays. A progressive trend of reduction over the control due to increasing doses of gamma rays was observed for germination, root and shoot lengths. A differential response in different parameters of *A. procera* under two moisture levels was also observed.

Seedling height was more sensitive than other parameters and decreased linearly with dose. Shoot length showed maximum reduction and lowest $ED_{50/20}$ values, i.e. 540 Gy for dry seeds and 135 Gy for presoaked seeds (Tables 1 & 3). Similarly, for fractionated irradiation, the $ED_{50/20}$ values were 620 Gy for dry seeds and 280 Gy for presoaked seeds. Root length was less affected and had $ED_{50/20}$ values of 770 Gy for dry seeds and 140 Gy for presoaked seeds under acute irradiation and 750 Gy and 290 Gy under fractionated irradiation respectively (Tables 2 & 3). The reason for the reduction in plant growth may be due to the increased synthesis of auxin (Deshmukh 1981), the slow rate of division of meristematic tissue of shoot and root apexes or chromosomal aberrations in irradiated cells (Rao & Rao 1983). Chromosomal damage and mitotic inhibition might have contributed to reduction in seedling growth because direct relationship between chromosomal aberration and injury has been reported (Evans & Sparrow 1961).

The total germination of seeds decreased due to acute and fractionated doses at both moisture levels and about 50 % reduction was observed at the highest dose. GV was very sensitive and showed highest reduction and lowest $ED_{50/20}$ values, i.e. 120 Gy and 690 Gy for acute irradiation of dry and presoaked seeds respectively (Tables 1 & 3). Much lower GV values were obtained for fractionated irradiation (Tables 2 & 3). The germination was delayed in irradiated seeds, thus values of GEI were influenced accordingly. This observation suggests that the irradiation reduced the energy of seeds and ultimately impeded the speed of germination (Kapoor 1981). The reduction in germination parameters may be due to the inhibitory effect of irradiation on embryonic cells, delayed and decreased mitotic activity of cells, or increased synthesis of active radicals (Selim *et al.* 1974). The disruption and disorganization of tunical layer, which is directly proportional to the intensity of gamma rays exposure, might have resulted in lethality (Lokesha *et al.* 1994). Survival of germinated seedlings due to irradiation of the seeds was not much affected.

In contrast to the above, cotyledon length and width showed a stimulatory effect of irradiation. Size of cotyledons was increased after gamma irradiation. The values of 10% stimulation observed for both the parameters under different modifying factors are presented in Table 3. The increase in size of cotyledons may be due to the accumulation of food and its underutilisation by the seedlings. Irradiation had an adverse effect on shoot length and may have resulted in the underutilisation of stored food material.

Table 1. Effect of gamma irradiation (acute) on germination and seedling parameters of *Albizia procera*, raised from dry (DS) and presoaked seeds (PS)

Dose in Gy		Replicate mean									
		Germination percentage	Germination value	Germination energy index	Survival percentage	Radicle length (cm)	Shoot length (cm)	Cotyledon length (mm)	Cotyledon width (mm)	Fresh weight (mg)	Dry weight (mg)
0	DS	88.00	131.30	57.79	98.86	1.68	4.73	8.31	5.35	140.22	20.40
	PS	80.00	117.50	53.33	100.00	2.39	4.12	8.11	5.42	153.75	19.60
20	DS	83.00	140.80	53.74	100.00	1.65	4.20**	8.04*	5.35	129.25	20.72
	PS	75.00	137.37	51.00	100.00	0.69	1.06	8.98	5.99	140.50	18.29
40	DS	80.00	140.30	55.00	100.00	1.05**	2.79**	9.20**	5.90**	142.00	20.90
	PS	70.00	120.25	47.25	97.29	0.70**	0.83**	5.89**	6.36**	126.50**	20.82
80	DS	72.00**	111.60	46.16**	97.21	0.81**	1.45**	9.32**	5.96**	134.57	22.35
	PS	58.00**	76.50**	38.25**	98.44	0.40**	0.84**	9.97**	6.36**	138.75*	21.50
120	DS	58.00**	65.30**	36.66**	94.89	0.60**	1.04**	9.33**	5.91**	130.98	18.85*
	PS	46.00**	50.75**	30.50**	98.08	0.32	0.73**	10.27**	6.48**	145.25	15.93**
160	DS	47.00**	41.70**	29.08**	91.29	0.65	0.97**	9.80**	6.12**	140.32**	22.32
	PS	41.00**	39.87**	27.25**	100.00	0.32**	0.67**	9.27**	6.40**	131.75**	15.75**
LSD	DS	8.57	28.81	5.04	11.25	0.25	0.27	0.23	0.32	28.14	2.87
(p = 0.05)	PS	5.35	26.51	6.26	8.42	0.14	0.08	0.42	0.22	14.70	2.56

* and ** = significant over control at 5% and 1% levels of significance respectively;
DS = dry seeds; PS = presoaked seeds.

Table 2. Effect of gamma irradiation (fractionated) on germination and seedling parameters of *Albizia procera*, raised from dry (DS) and presoaked seeds (PS)

Dose in Gy		Replicate mean									
		Germination percentage	Germination value	Germination energy index	Survival percentage	Radicle length (cm)	Shoot length (cm)	Cotyledon length (mm)	Cotyledon width (mm)	Fresh weight (mg)	Dry weight (mg)
0	DS	84.50	134.90	55.75	94.26	1.78	5.49	7.52	4.86	132.75	18.21
	PS	85.00	153.87	55.00	100.00	1.85	3.85	7.58	5.63	146.75	20.75
100 + 100	DS	80.00	132.90	53.91	96.26	1.55	4.62*	8.17	5.33	143.75	21.06
	PS	73.00**	118.62**	47.25**	100.00	1.23**	2.49	8.75	5.90	146.25	21.18
200 + 200	DS	79.00	119.40	53.60	88.36	1.04**	3.03**	8.39*	5.48*	138.00	10.97
	PS	70.00**	115.00	45.10**	100.00	0.52*	1.07**	9.30**	6.08**	132.25*	18.75
400 + 400	DS	62.00**	50.20**	37.37**	95.38	0.85**	2.30**	8.59**	5.69**	126.75	19.90
	PS	64.00**	84.12**	40.25**	94.80	0.52**	0.95**	9.25**	6.10**	131.50*	21.54
600 + 600	DS	57.00**	60.05**	34.91**	97.06	0.90**	2.07**	8.20	5.81**	138.75	22.48
	PS	52.00**	57.25**	33.50**	96.13	0.34**	0.86**	9.55**	6.18**	129.75*	18.92
800 + 800	DS	45.00**	30.50**	27.33**	93.56	0.52**	1.09**	9.13**	6.10**	128.25	21.18*
	PS	40.00**	37.67**	25.00**	100.00	0.41**	0.80**	9.60**	6.45**	124.50**	19.91
LSD	DS	5.60	28.42	8.53	14.73	0.34	0.69	0.86	0.52	15.72	2.92
(p = 0.05)	PS	5.66	35.01	7.22	7.69	0.26	0.62	0.55	0.34	13.91	3.16

* and ** = significant over control at 5% and 1% levels of significance respectively;
DS = dry seeds; PS = presoaked seeds.

Table 3. Mean square and $ED_{50/20}$ values of various parameters of *Albizia procera* seedlings raised from dry and presoaked seeds, irradiated with acute and fractionated doses of gamma rays

Source of variation	Degree of freedom	Mean Square									
		Germination percentage	Germination value	Germination energy index	Survival percentage	Radicle length	Shoot length	Cotyledon length	Cotyledon width	Fresh weight	Dry weight
1. Acute doses of gamma rays											
Replications	DS 3	17.570	295.350	4.008	143.820	0.009	0.088	0.241	0.027	158.512	19.963
	PS 3	8.350	222.820	4.261	22.380	0.009	0.054	0.049	0.087	63.389	2.120
Treatments	DS 5	468.090***	7 076.630***	521.920***	102.750	0.929***	10.764***	1.839***	0.4324***	116.077	9.837
	PS 5	370.670	11 524.150	519.380***	32.300	2.550***	7.276***	2.475***	0.656***	373.367*	23.787***
Error	DS15	32.340	365.650	11.190	55.710	0.028	0.0318	0.110	0.046	348.700	3.628
	PS 15	12.600	309.520	17.290	31.230	0.009	0.0031	0.077	0.021	95.256	2.900
$ED_{50/20}$ in Gy	DS	ED_{20}- 840	1200	1600	NA	771	540	+370[1]	+400[1]	NA	NA
	PS	ED_{20}- 600	690	1580	NA	140	135	+190[1]	+200[2]	NA	ED_{20}-1600
2. Fractionated doses of gamma rays											
Replications	DS 3	28.033	542.975	43.117	43.440	0.027	0.075	0.194	0.032	135.375	2.167
	PS 3	39.695	1180.585	64.556	9.093	0.022	0.361	0.100	0.140	73.000	2.546
Treatments	DS 5	391.420***	8631.223***	586.097***	70.721	0.894***	11.022***	1.112*	0.733***	175.242	8.531
	PS 5	405.663***	7401.525***	451.100***	98.620*	1.438***	6.194***	2.270***	0.305**	337.667*	5.502
Error	DS15	13.810	355.813	32.071	95.520	0.050	0.213	0.327	0.119	108.775	3.763
	PS 15	14.094	539.788	22.989	26.038	0.030	0.170	0.131	0.051	85.200	4.469
$ED_{50/20}$ in Gy	DS	ED_{20}- 670	705	1580	NA	750	620	+ 1570[1]	+1254[1]	NA	NA
	PS	1520	910	1490	NA	290	280	+ 340[1]	+1280[2]	NA	NA

*, **, *** = significant at 5%, 1% and 0.1% levels of probability respectively;
[1] & [2] = 20% and 10% stimulation in Gy, NA = not achieved, DS = dry seeds, PS = presoaked seeds.

Fresh and dry weights of seedlings showed an irregular trend of reduction or stimulation. However, slight reduction in fresh weight was observed in acute and fractionated modes of irradiation to presoaked seeds. As the biomass is directly related to the growth of plants, reduction observed in growth was reflected in the reduction of fresh and dry weights of seedlings. The increase in fresh and dry weights observed in some treatment may be due to increase in cotyledon size and weight.

Bridges and laggards at mitotic anaphase were observed in almost all the treatments and frequency of abnormal cells increased with increasing dose of gamma rays (Table 4). The mitotic cycle was delayed in treated material. Delay in mitosis may be due to the formation of antimitotic substances or physical changes in protoplasts, and bridge formation may be due to stickiness or non-separation of chromosomes.

Table 4. Chromosomal abnormalities observed in the root tips of *Albizia procera* seedlings raised from dry and presoaked seeds, irradiated with acute and fractionated doses of gamma rays

Dose in Gy	Acute irradiation				Fractionated irradiation			
	Dry seeds		Presoaked seeds		Dry seeds		Presoaked seeds	
	Total cells observed	% of abnormal cells	Total cells observed	% of abnormal cells	Total cells observed	% of abnormal cells	Total cells observed	% of abnormal cells
0	102	0.00	118	0.00	137	0.00	129	0.00
200	122	0.82	276	0.36	161	0.00	267	0.00
400	224	0.89	181	0.55	171	0.59	173	0.58
800	138	0.73	205	0.49	302	0.66	136	0.74
1200	318	0.63	191	1.05	187	1.07	251	1.19
1600	96	1.04	212	0.94	102	2.94	164	-

- = not observed due to minute size of root-tip.

In the present study, presoaked seeds were more sensitive than air dry seeds. Similar results have also been reported by Kapoor (1981), Renu and Kapoor (1989a, b) and Bhandari (1993). Increase in radiosensitivity due to moisture has been attributed to increase in metabolic activity (Caldecott 1955) and synthesis of germination inhibitors (Barton & Salt 1948). Conger (1961) postulated that at low moisture levels the seed embryo is like a crystalline solid and the members of a pair of radicals formed due to irradiation could recombine. As the moisture level increases the radicals are more likely to react with the genetic material and produce more mutations.

This species was slightly more sensitive to fractionated doses of gamma rays than to the acute doses. Similar results were reported by Kapoor (1981), Renu and Kapoor (1989a,b) and Singh and Paliwal (1987) while studying the radiosensitivity of *Albizia lebbek*, *Eucalyptus* hybrids and *Pinus roxburghii*.

The high $ED_{50/20}$ values observed for different parameters of this species agree with the results of Bhandari (1993) and Goel (1987), who have also reported high $ED_{50/20}$ doses for *Dalbergia sissoo, Acacia auriculiformis, A. nilotica,* and *Prosopis juliflora.* The small size of the chromosomes or the presence of radio-protective agents *in situ* might have reduced the sensitivity of this species. On the basis of these studies it is inferred that germination value and seedling height are useful parameters to assess the radiosensitivity of a species.

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References

BARTON, L.V. & SALT, M.L. 1948. Growth inhibitors in seeds. *Contrib. Boyce Thompson Inst. Ithaca, New York* 15 : 259 - 278.

BHANDARI, M. 1993. Radio-sensitivity of *Dalbergia sissoo* (Roxb.). I. Effect of gamma rays vis-a-vis seed extract on seed germination and seedling growth. *Annals of Forestry* 1(2) : 160 - 164.

CALDECOTT, R.S. 1955. Reduction in X-rays sensitivity of seeds by hydration. *Nature* 176 : 306.

CONGER, A.D. 1961. Biological after effects and long lived free radicals in irradiated seeds. Recovery of cells from injury. *Journal of Cellular Composition and Physiology* (Supplement) 58 : 27 - 32.

CZABATOR, F.J. 1962. Germination value: an index combining speed and completeness of pine seed germination. *Forest Science* 8(4) : 386 - 396.

DESHMUKH, S.V. 1981. Effects of gamma rays on R_1 parameters in *Antheum sowa,* Kurz. Pp. 353-357 in Manna, G.K. & Sinha, V. (Eds.) *Perspective in Cytology and Genetics. Proceedings of 3rd All India Congress on Cytogenetics and Genetics, New Delhi.*

EVANS, H.J. & SPARROW, A.H. 1961. Nuclear factors effecting radiosensitivity. II. Dependence of nuclear and chromosomal structure and organization. *Brookhaven Symp. Bio.* 14 : 101 - 127.

GOEL, V. L. 1 987. Radiosensitivity of some firewood trees. *Indian Journal of Forestry* 10(3) : 167 - 172.

GROESE, R.J. & ZIMMER, W.J. 1958. Some laboratory germination responses of the seeds of river red gum, *Eucalyptus camaldulansis* Dehn. *Australian Journal of Botany* 6 : 129 - 153.

GUSTAFSSON, A. 1960. Polyploidy and mutagenesis in forest tree breeding. Pp. 793-805 in *Proceedings of Vth World Forestry Congress, Seattle, 2.*

KAPOOR, M.L. 1981. Studies on the relative sensitivity on some selected forest tree species to physical and chemical mutagens. D. Phil. thesis, Garhwal University, Sri Nagar, India. 119 pp.

LOKESHA, R., VASUDEVA, R., SASHIDHAR, H.E. & REDDY, A.N.Y. 1994. Radio-sensitivity of *Bambusa arundinacea* to gamma rays. *Journal of Tropical Forest Science* 6(4) : 444 - 450.

MICKE, A. 1984. Induced mutations in plant breeding. *Radiation Botany* 15 : 84.

RAO, S.R.M. & RAO, D. 1983. Gamma rays induced physiological variabilities in black gram (*Vigna mungo*). *Indian Journal of Botany* 6(1) : 40 - 44.

RENU, S. & KAPOOR, M.L. 1989 a. Biological effects of combine treatments of ethyl methanesulphonate and gamma rays on *Eucalyptus* hybrid. *Indian Journal of Forestry* 12(3): 224 - 230.

RENU, S. & KAPOOR, M.L. 1989b. Differential response of *Eucalyptus tereticornis* x *E. camaldulensis* to continuous and fractionated doses of gamma rays at two moisture levels. *Indian Journal of Forestry* 12(4) : 258 - 264.

SELIM, A.R., HUSSEIN, H.A.S. & EL. SHAWOF, I.S. 1974. EMS and gamma rays induced mutation in *Pisum sativum.* II. Effects of EMS and gamma rays on M2 generation seedlings, height and fertility. *Egyption Journal of Genetics and Cytology* 3 : 172 - 192.

SINGH, S.S. & PALIWAL, G.S. 1987. Sensitivity of *Albizia lebbek* seeds to gamma rays. *Indian Forester* 113(7): 49 - 50.

SPARROW, A.H., ROGERS, A.H. & SCHWEMMER, S.S. 1968. Radiosensitivity studies with woody plants I. Acute irradiation data on 28 species and prediction for 109 species. *Radiation Botany* 8 : 149 - 186.