Theor. Appl. Genet. 59, 11-15 (1981)

X-ray Irradiation of Excised Embryos of Mesta (*Hibiscus cannabinus* L. and *H. sabdariffa* L.)

A. Shome
Jute Agricultural Research Institute, Barrackpore, West Bengal (India)

Summary. Excised embryos of *Hibiscus* spp. were treated with 1 kR to 6 kR of X-ray. Results indicate that germination was unaffected at this level of employed doses in both species, which in turn implies that the factors responsible for inhibition of germination are not present in the embryo. LD_{50} values differed between varieties and species. Early varieties of both species were more sensitive to radiation than late varieties. Strikingly similar effects were observed for the varieties with smaller embryos over those with larger ones. Allopolyploid *H. sabdariffa* (2n = 72) was more susceptible than diploid *H. cannabinus* (2n = 36).

Differences in mutation frequency exist between species with different levels of ploidy and between varieties within the same species. Most of the HC mesta varieties yielded higher mutation frequencies than those of HS mesta. Optimal dose for triggering mutations in all varieties (except the chlorophyll mutation variety of HC mesta) of the two species lies within a narrow range of 1 kR to 2 kR. Cent per cent seedling abnormalities is concomitant to LD_{50}; nevertheless, optimum dose for mutation frequency is independent of LD_{50}. Hence, the response should be viewed in terms of respective genotype. The advantages of the embryo irradiation technique are mentioned.

Key words: Germination – Seeding lethality – Abnormalities – LD_{50} value – Mutation frequency

Introduction

In bast (stem) fibre crops previous investigations on radiosensitivity and induction of mutation have been limited to dry seed irradiation with high doses of X-rays (Kundu et al. 1961; Singh and Mitra 1967; Singh et al. 1973). Excluding my earlier approach (Shome 1979) there has been no rigorous attempt to study the effect of X-rays on excised mesta embryos. The following is a detailed account of a study designed to analyse the relative radiosensitivities of some genotypes of mesta (*Hibiscus cannabinus* L. and *H. sabdariffa* L.). As an outgrowth of this study the mutation frequencies of the excised embryos were critically examined.

Materials and Methods

The experimental materials consisted of five inbreds (MT 15, MT 166, MT 749, HC 583, HC 867) of HC mesta (*H. cannabinus*; a diploid, 2n = 36); four inbreds ('Roselle early', RT 1 (NBr), RT 2, AMV 1); two hybrids, viz. HS 4288 (RT 1 Br × RT 2), HS 7910 (RT 1 NBr × HS 4288) of HS mesta (*H. sabdariffa*; appeared to be an allopolyploid, 2n = 4× = 72, as mentioned by Menzel 1964). Of the different varieties, MT 15 of HC mesta and 'Roselle early' of HS mesta are early varieties; the others are late varieties. Seeds of these varieties were soaked in distilled water for 4 hr and embryos were excised by the technique previously described (Shome 1979). The light-cream coloured excised embryos were treated in plastic petri plates to 1 kR to 6 kR of X-rays by a 100 kV, 11 mA machine at a dose rate – 1600 R/min. The embryos were kept at a distance of 10 cm from the tube source. The final doses were based on preliminary observations made earlier from a wide range of doses administered to embryos. In this experiment, a total of 7,700 embryos were excised and in all cases (varieties) one hundred embryos were treated with each dose and kept in petri plates in four replications. The cultures were maintained for 48 hr in petri plates at 30°C ± 2°C in 12 hr fluorescent light followed by transfer to soil, as done earlier (Shome 1979). Counts were made on seedling survival on the 7th, 14th, and 21st day after irradiation and LD_{50} values were calculated from 21 day field culture. Final records on adult plants were noted at the time of harvest (M_1). The radiation damage expressed was based on seedling survival (on 21st day) as per cent of their respective controls. The selfed seeds of each M_1 plant were carefully collected to raise the M_2. The M_2 was raised as M_1 progeny rows. The seeds were harvested from individual plants of the M_2 lines yielding mutations for further confirmation in the M_3 generation. The frequency of mutation was calculated according to the method of Bhaduri and Shome (1969).

0040-5752/81/0059/0011/$ 1.00

Results and Discussion

Germination

Germination (elongation of radicale and hypocotyl, associated with greening and unfolding of cotyledons, are the parameters of germination) was unaffected, irrespective of treatment, in both species. Moreover, germination was hastened, (the intact seeds required 48 to 72 hr to germinate and the excised embryo germinated within 24 hr). A 100% germination of excised embryo implicated that factors responsible for inhibition of germination are not present in the embryo. This is in line with the observation made for excised rice embryos in vitro (Bhaduri and Shome 1969). The threshold value of X-ray doses, beyond which the injurious effect of X-rays on germination would be apparent, remains to be analysed in future investigations.

Seedling Lethality

Injurious effects of X-rays on seedlings was conspicuous from the third to fifth day of culture in the forms of a shrunken appearance of the lower part of the shoot, drooping of cotyledons, root degeneration, etc. Seedling lethality could be seen from the sixth day onwards. It is interesting to note that this characteristic response was irrespective of varieties and species as well as the doses applied. The seedling lethality, in general, increased with increasing level of radiation (Table 1). However, it is also evident that many established seedlings of the treated progenies died even in field culture (after 21 days from irradiation) as against controls, indicating that the injurious effects of X-rays on excised embryos as well as on seeds are identical.

Table 1. Seedling survival on the 21st day as percentages of controls[a] following X-ray treatments of excised embryos of mesta

Species and Varieties	Treatments					
	1 kR	2 kR	3kR	4kR	5kR	6kR
H. cannabinus						
MT 15	86.3 (66.1)[b]	85.8 (47.0)	77.1 (45.0)	69.5 (24.2)	12.5 (0.0)	2.0 (0.0)
MT 749	86.9 (100.0)	78.6 (73.4)	76.0 (62.0)	71.2 (25.3)	52.0 (12.6)	7.8 (2.5)
MT 166	88.5 (77.7)	87.5 (61.0)	81.0 (48.8)	76.4 (42.2)	68.6 (10.0)	45.8 (2.2)
HC 867	91.8 (86.4)	90.6 (79.2)	89.6 (76.7)	88.3 (62.1)	70.9 (14.6)	46.5 (9.7)
HC 583	88.0 (85.3)	85.1 (75.2)	84.6 (61.7)	83.3 (59.5)	64.0 (32.5)	64.0 (22.4)
H. sabdariffa						
'Roselle early'	95.7 (74.8)	48.9 (32.5)	12.7 (0.0)	c	c	c
RT 1	96.7 (67.9)	86.9 (65.2)	83.6 (64.1)	69.1 (12.8)	30.4 (2.5)	15.2 (0.0)
RT 2	98.7 (98.5)	90.9 (60.0)	87.0 (47.0)	14.8 (0.0)	c	c
AMV 1	95.6 (95.8)	87.0 (80.5)	65.5 (34.7)	31.1 (0.0)	12.0 (0.0)	c
HS 7910	75.0 (86.0)	61.2 (55.0)	50.0 (40.0)	47.5 (0.0)	7.5 (0.0)	c
HS 4288	91.0 (100.0)	91.0 (93.9)	71.7 (51.5)	70.5 (28.7)	69.2 (10.6)	21.7 (0.0)

[a] Value obtained after dividing mean of survival of treatments by mean survival of respective controls x 100
[b] Figures in parentheses indicate survival of plants at harvest (per cent of control)
[c] No survival beyond 2 weeks

Table 2. Morphological abnormalities (%) at seedling stage (on the 14th day) following X-ray treatments of excised embryos of mesta[a]

Dose in kR	Varieties of *H. cannabinus*					Varieties of *H. sabdariffa*					
	MT 15	MT 749	MT 166	HC 867	HC 583	'Roselle early'	RT 1	RT 2	AMV 1	HS 7910	HS 4288
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	4.0	10.4	0.0	3.8	0.0	80.5	61.5	13.0	9.10	17.5	32.4
2	23.32	28.9	3.8	18.4	12.5	100.0	97.0	91.8	78.3	90.3	53.3
3	43.38	46.7	46.4	59.2	48.0	100.0	97.0	98.0	96.4	100.0	75.7
4	60.0	86.0	81.4	68.4	80.4	100.0	100.0	100.0	100.0	100.0	91.6
5	100.0	100.0	90.0	94.5	89.3	100.0	100.0	100.0	100.0	100.0	100.0
6	100.0	100.0	100.0	100.0	94.2	100.0	100.0	100.0	100.0	100.0	100.0

[a] Values of control are zero, hence mean values of irradiated populations of both the species are increased (+) values over control

Seedling Abnormalities

Prior to transplantation, X-ray injury – root tip burning, bifurcation, hard texture and brown spots, was restricted to the roots only. After transplantation, and from the sixth day onwards, seedling anomalies involving cotyledons, leaves and shoots were distinctly visible in both species, even at the lowest level of radiation. Furthermore, seedling abnormalities increased with increasing doses of X-rays (Table 2). However, different M_1 abnormalities of both species failed to appear in the progenies of later generations (M_2, M_3), indicating that they were nonheritable.

LD_{50}

Sub-lethal doses differed between varieties and species (Tables 1 and 3). Within HC mesta, HC 583 was found to be more resistant (LD_{50} above 6 kR) as compared to other varieties. Similarly, in HS mesta, HS 4288 (LD_{50} occurred between 5 kR to 6 kR) was more resistant than other varieties. It is of interest to note that early varieties of both species are more susceptible than late varieties. Results also revealed that, between species, diploid *H. cannabinus* (2n = 36) was more resistant than allopolyploid *H. sabdariffa* (2n = 4X = 72).

Bacq and Alexander (1961) pointed out that the degree of radiosensitivity was genetically controlled. The intra- and inter-specific differences of the radiosensitivities in the present case indicate that they are related to genotypic differences. However, in addition to genotype, kernel size and embryo size often determine the degree of radiosensitivity of a variety; i.e. varieties with smaller kernels and embryos were more susceptible to X-irradiation (Mikaelson and Halvorsen 1953; Bhaduri and Shome 1969; Shome and Bhaduri 1980). It may be mentioned here that, in general, HC mesta has larger embryos than HS mesta. Therefore, increased radiosensitivity of the latter species (HS mesta) may be coupled with the smaller embryo size. The above hypothesis is further supported by the present observation that both an early variety (MT 15) of HC mesta as well as 'Roselle early' of HS mesta have smaller embryos and were more susceptible to X-irradiation.

Table 3. Dose required for 50% seedling lethality (LD_{50}) and optimal level of X-rays for induction of mutation (other than chlorophyll mutation) in mesta

Species and varieties	LD_{50} (on 21st day)	Optimum dose for mutation
H. cannabinus		
MT 15	4 – 5 kR (5 kR)[a]	1 kR
MT 749	~ 5 kR (5 kR)	1 kR
MT 166	5 – 6 kR (6 kR)	1 kR
HC 867	5 – 6 kR (6 kR)	1 kR
HC 583	> 6 kR (above 6 kR)	2 kR
H. sabdariffa		
'Roselle early'	~ 2 kR (2 kR)[a]	[b]
RT 1	4 – 5 kR (4 kR)	2 kR
RT 2	~ 3.5 kR (4 kR)	1 kR
AMV 1	~ 3.5 kR (4 kR)	1 kR
HS 7910	~ 3.0 kR (3 kR)	1 kR
HS 4288	5 – 6 kR (5 kR)	2 kR

[a] Figures in parentheses indicate the levels of X-rays where 100% seedling abnormalities were noted

[b] No mutation

Mutation Frequency

Mericle and Mericle (1962) obtained the highest mutation rate as well as larger 'isomutant carrying sectors' in a R_2 generation following proembryo irradiation. Bhaduri and Shome (1969) indicated that frequency of polygenic mutations was higher in cases of irradiated embryos where lower doses of X-rays determined the LD_{50} value. Singh and Mitra (1967) induced heritable variants only in the case of HC mesta after treating the dry seeds of HC and HS mesta with 20 kR to 140 kR of X-ray. In this study with excised embryos, mutation was induced in both species of *Hibiscus* with low levels of X-rays (1 kR to 6 kR). Furthermore, the mutant sectors obtained through embryo treatments in the M_2 were bigger when compared to those of the seed treatments (Singh and Mitra 1967).

With respect to chlorophyll mutation, 'lethal yellow' surviving up to 28 to 30 days appeared only in two varieties of HC mesta and two hybrids of HS mesta in a ratio of 3 : 1 (lethal yellow : normal), implicating that it is

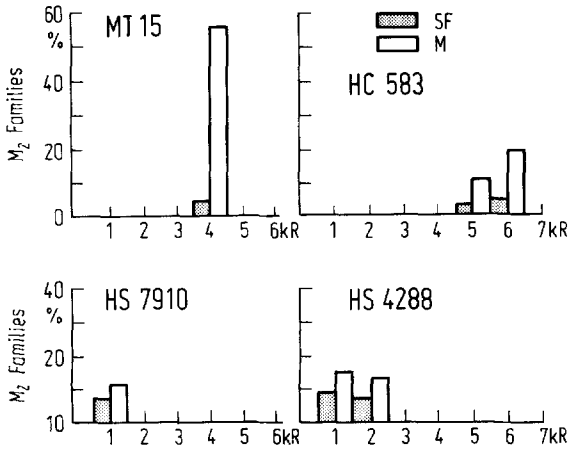

Fig. 1. Chlorophyll mutation frequencies in *Hibiscus cannabinus* and *H. sabdariffa*. SF = % of M_1 families segregating for mutants; M = % of mutations per M_1 families

monogenic. In HC mesta, chlorophyll mutation was scored from 4 kR onwards while in the case of HS mesta it was scored from lower doses (Fig. 1). Hence, except for the type of mutation, the two species reflected a differential response either in terms of mutation frequency or optimum dose for induction of chlorophyll mutation which may be due to factors controlling chlorophyll development (Tsunekawi and Heyne 1959; Swaminathan 1965a).

In addition to chlorophyll mutation, macro-mutations (bred true to mutant characters in M_3) mostly affecting qualitative characters were also isolated in the M_2 generation. Results showed that considerable differences in mutation frequency exist between varieties within the same species and between species of the different ploidy level. Thus HC 583 from HC mesta and RT_2 from HS mesta yielded higher mutation frequencies as compared to the other varieties (Figs. 2, 3). Between species, most of the varieties of diploid HC mesta showed higher rates of mutation as compared to allopolyploid HS mesta. It also emerged that mutation frequencies decreased in all the varieties of HC mesta (except at 2 kR in HC 583) with increasing levels of X-rays. However, in HS mesta (except RT_2), the rate of mutation was not always decreased with increasing dose of radiation.

It is of significant interest to note that the optimal dose for triggering mutation in all the varieties (barring chlorophyll mutation of HC mesta) of the two species varies within a narrow range of 1 kR to 2 kR (Table 3; Figs. 2, 3). The present study further revealed that 100% seedling abnormalities is concomitant to LD_{50}; nevertheless, optimum dose for mutation frequency is independent to LD_{50} value. Hence, the response should be viewed in terms of respective genotype.

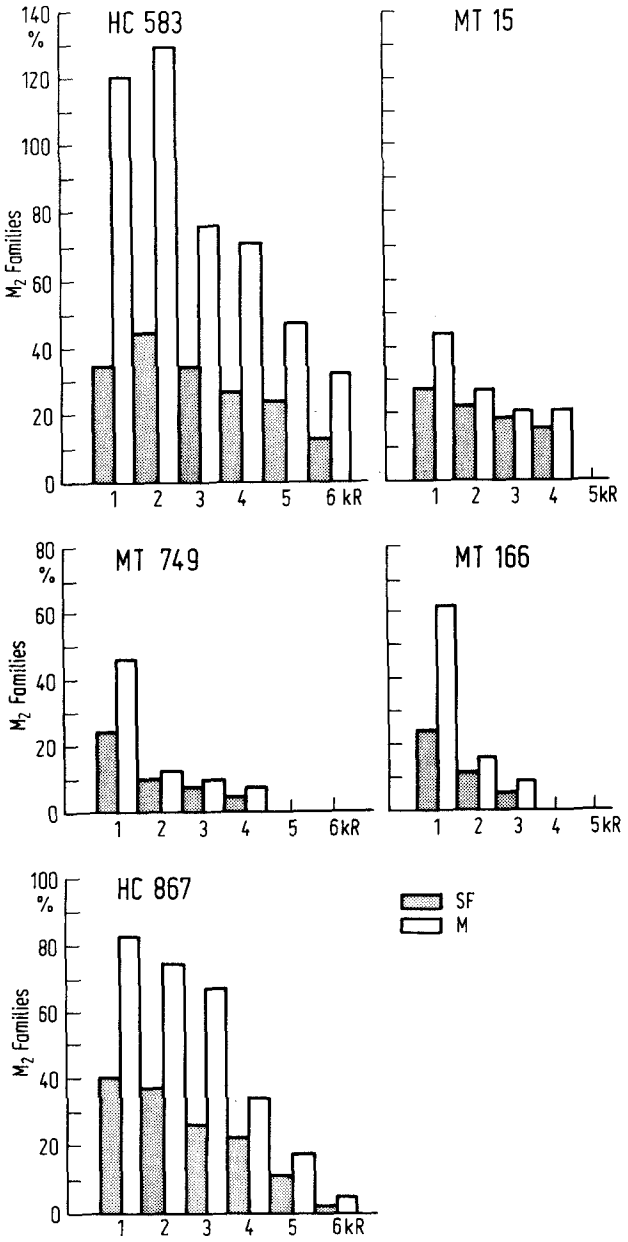

Fig. 2. Macro-mutations frequencies in *H. cannabinus*. SF = % of M_1 families segregating for mutants; M = % of mutations per M_1 families

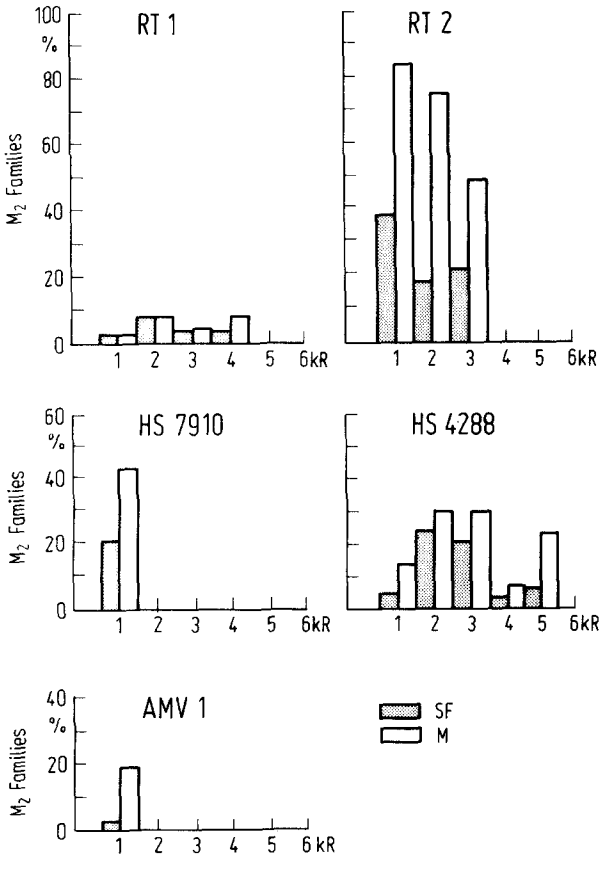

Fig. 3. Macro-mutations frequencies in *H. sabdariffa*, SF = % of M_1 families segregating for mutants; M = % of mutations per M_1 families

This study generates an efficient technique for obtaining higher mutation frequencies in bast (stem) fibre crops. Eventually, the probability of evolving beneficial mutations encompassing polygenic systems would increase using this technique because comparatively lower doses of X-rays could be effectively employed for induction of mutation than that used on whole seeds as done by earlier workers (Singh and Mitra 1967).

Acknowledgement

I wish to thank Drs. S.L. Basak, D.P. Singh and S.K. Hazra for their encouragement and helpful suggestions and Dr. P.N. Bhaduri (Emeritus Scientist, ICAR) for his interest in the investigation.

Literature

Bacq, Z.M.; Alexander, P. (1961): Fundamentals of Radiobiology, pp. 50-100. Oxford: Pergamon Press

Bhaduri, P.N.; Shome, A. (1969): X-ray irradiation to dissected embryos of rice and their mutation spectrum. Curr. Sci. 38, 297-298

Kundu, B.C.; Ghosh, K.L.; Sarma, M.S. (1961): Studies on the effects of X-irradiation in *Corchorus capsularis* L. and *C. olitorius*. Genetica 32, 51-73

Menzel, M.Y. (1964): Cytological studies with Kenaf. 2nd Intl. Kenaf Conf. Palm Beach, Fl. pp. 134-138

Mericle, L.W.; Mericle, R.P. (1962): Mutation induction by proembryo irradiation. Radiat. Bot. 1, 195-202

Mikaelson, K.; Halverson, H. (1953): Experiments on the respiration of X-irradiated barley seeds. Physiol. Plantarum 6, 873-879

Singh, D.P.; Mitra, G.C. (1967): X-irradiation effects on *Hibiscus cannabinus* L. and *Hibiscus sabdariffa* L. Genet. Agrar. 21, 86-100

Singh, D.P.; Sarma, B.K.; Banerjee, S.C. (1973): X-ray induced mutation in Jute (*Corchorus capsularis* L. and *Corchorus olitorius* L.). Genet. Agrar. 27, 115-147

Shome, A. (1979): Radiosensitivity of excised embryos of mutant in *Hibiscus cannabinus* L. Bangladesh J. Bot. 8, 98-105

Shome, A.; Bhaduri, P.N. (1980): Radiosensitivity of the excised rice embryo. Cytologia (in press)

Swaminathan, M.S. (1965a): A comparison of mutation induction in diploids and polyploids. In: The Use of Induced Mutations in Plant Breeding, pp 619-641. Rep. FAO/IAEA Tech. Meeting, Rome. Oxford: Pergamon Press

Tsunewaki, K.; Heyne, E.G. (1959): Radiological study of wheat monosomics. I and II. Genetics 44, 933-46; 947-954

Received May 15, 1980
Accepted July 11, 1980
Communicated by K. Tsunewaki

Dr. A. Shome
Plant Breeding and Genetics Division
Jute Agricultural Research Institute
Barrackpore 743101
24 Parganas, W.B. (India)