

POLLEN IRRADIATION IN COTTON (*GOSSYPIUM HIRSUTUM* L.)

M. ASLAM, R.M.S. IQBAL, M.B. CHAUDHRY
AND A.A. BANDESHA

*Nuclear Institute for Agriculture and Biology,
Faisalabad, Pakistan.*

Abstract

Response of cotton pollen to 0.5, 1.0, 2 and 5 kR of gamma-rays prior to cross pollination was determined in various cross-combinations. A low dose of 0.5 kR applied to pollen enhanced boll set and seeds per pollination, whereas pollen irradiation with higher doses of 2.0 to 5.0 kR, before cross pollinations showed a sharp decline in boll set and seed production compared to control treatment. M_1 generation studies showed that higher doses of 2.0 to 5.0 kR of pollen radiation decreased the emergence and survival rate whereas phenotypic and fertility changes significantly increased at 2.0 kR. Varietal response to pollen radiation was non significant for phenotypic changes but significant for fertility changes. Treatment of pollen with 0.5 to 1.0 kR of gamma-rays is suitable for inducing useful genetic variability in cotton.

Introduction

Exposure of seed to ionizing radiations has been used to generate genetic variability in different crop species and many plant breeding programmes have demonstrated the feasibility of irradiation plus selection as a direct method of varietal improvement (Al-didi, 1965; Carnelius, 1973; Micke & Donini, 1982; Micke *et al.*, 1987; Iqbal *et al.*, 1991). The work on *Nicotiana* have shown that irradiation of pollen may provide a different but equally valuable tool for plant breeders (Pandey, 1978; Jinks *et al.*, 1981). In cotton pollen irradiation to induce mutations had been reported (Pate & Duncan, 1963; Krishnaswami & Kothandaraman, 1976). The present studies were carried out to evaluate the response of cotton pollen to gamma radiation to determine a suitable radiation dose to be applied to pollen for cotton improvement.

Materials and Methods

Three exotic cotton varieties/lines viz., USA-35, USA-37, RQB-62 and one most widely grown local cotton variety NIAB-78 were used. NIAB-78 was kept as female parent while USA-35, USA-37 and RQB-62 were used as pollen parent. Male and female parents were grown from the selfed seeds to develop about 100 healthy plants. Flower buds at suitable maturity of female parent were emasculated in the afternoon and covered with thin paper bags. Then in the morning male parent pollen was collected from the covered flowers after anthesis and irradiated with gamma-rays at 0.5 (T_1), 1.0 (T_2), 2.0 (T_3) and 5.0 (T_4) kR from a ^{60}Co radiation source. Non-irradiated pollen was also collected. Emasculated flowers were pollinated with irradiated pollen

Table 1. Number of bolls and seeds produced in different crosses in cotton with irradiated pollen (1989).

Irradiation treatments	Bolls Produced (No.)				Seeds Produced (No.)			
	Cross-1	Cross-2	Cross-3	Mean	Cross-1	Cross-2	Cross-3	Mean
To - 0 kR	8.7 b	8.7 b	8.3 b	8.7 B	220.3 b	218.7 b	220.0	219.7 B
T1 -0.5 kR	10.7 a	10.3 a	10.7 a	10.6 A	289.0 a	295.0 a	291.3 a	291.8 A
T2 -1.0 kR	7.0 c	6.7 c	6.7 c	6.8 C	112.7 c	102.7 c	106.3 c	107.2 C
T3 -2.0 kR	4.0 d	4.3 d	4.0 d	4.1 D	49.7 d	54.7 d	48.7 d	51.0 D
T4 -5.0 kR	1.0 e	1.3 e	1.0 e	1.1 E	3.3 e	3.3 e	2.7 e	3.1 E
Mean	6.3 A	6.3 A	6.1 A		135.0 A	134.9 A	133.8 A	
Crosses x Doses	N.S.				N.S.			

Means followed by different letters (capital and small) differ significantly as determined by DMR Test.

N.S. = Non-significant,

Cross-1 = NIAB-78 X USA-35,

Cross-2 = NIAB-78 X USA-37,

Cross-3 = NIAB-78 X RQB-62

and rebagged to prevent uncontrolled crossing. Emasculated flowers were also pollinated with un-treated pollen and kept as control (T_0). At least 45 pollinations were made for each treatment. Crossed plants were fully protected from the adverse factors like drought and insect damage which effect the boll development. Bolls developed from the crossed flowers were harvested and number of seeds counted. These seeds designated as the zero generation of radiation treatment (M_0). The experiment was repeated during 1990 crop season with low doses of pollen radiation. M_0 seeds obtained in 1989 were studied as M_1 generation during 1990. Almost equal number of seeds i.e., one third seeds from the T_0 treatment and half of the seeds from T_1 treatment and all of the seeds from the other treatments from the 3 cross-combinations were planted in the field in single seed hills. Control and M_1 plants were examined and survival percentage, phenotypic changes and fertility changes for each individual plant were determined. Departures from control for leaf shape, boll type and general plant type characteristics were considered to be phenotypic changes. Plants producing less than 5 bolls/less than 100 open pollinated seeds per plant were considered having fertility changes (Aslam & Stelly, 1992; Pate & Duncan, 1963). The data collected were statistically analysed (Steel & Torrie, 1980).

Results

Number of bolls and seed produced in various cross combinations i.e., NIAB-78xUSA-35, NIAB-78xUSA-37 and NIAB-78xRQB-62 with irradiated pollen during

1989 are presented in Table 1. The boll set and seed production in various treatments differed significantly in all the cross-combinations. Largest number of bolls and seeds were produced from T₁ treatment. Reduced boll set and seed were obtained from T₂, T₃ and T₄ treatments. However, a significant reduction in boll set and seed production were observed in T₃ and T₄ treatments. No healthy or filled seeds were obtained from T₄ treatment. All the varieties showed similar response to gamma-radiation. However there were significant differences in boll set and seed production between the radiation doses applied to pollen. The experiment was repeated in 1990 with lower doses of gamma-rays and the results showed no inconsistency (Table 2).

Number of seed planted and percentage of matured plants, phenotypic changes and fertility changes in control and M₁ population are presented in Table 3. Emergence and survival percentage in T₁ treatment was significantly greater than the T₀ and T₂ treatments while T₀ and T₂ treatments showed similarity for emergence and survival rate. The emergence and survival rate reduced in T₃ treatment in all the cross-combinations while in T₄ treatment none of seed could germinate from all the crosses. Increasing the treatment rate from T₁ to T₃ level, showed a progressive increase in phenotypic changes reaching to 94-97 % in T₃ treatments. In all the cross combinations the fertility changes were greatest in T₃ treatment (94-97%) while about 18-27 % of the plants from T₁ and T₂ treatments showed fertility changes. Different radiation doses applied to pollen before cross pollinations have shown significant difference for phenotypic changes and fertility changes. However the varietal response was non significant for phenotypic changes and significant for fertility changes.

Table 2. Number of bolls and seeds produced in different crosses in cotton with irradiated pollen (1990).

Irradiation treatments	Bolls Produced (No.)				Seeds Produced (No.)			
	Cross-1	Cross-2	Cross-3	Mean	Cross-1	Cross-2	Cross-3	Mean
T1-0 kR	8.3 b	8.3 b	8.7 b	8.4 B	225.3 b	215.0 b	223.3 b	221.2 B
T2-0.5 kR	10.3 a	10.7 a	11.0 a	10.7 A	287.3 a	294.3 a	293.0 a	291.6 A
T3-1.0 kR	6.7 c	6.3 c	6.7 c	6.6 C	105.3 c	104.0 c	118.3 c	109.2 C
T4-2.0 kR	4.3 d	4.0 d	4.0 d	4.1 D	46.0 d	44.0 d	43.3 d	44.4 D
Mean	7.4 A	7.3 A	7.6 A		166.0 A	164.3 A	169.5 A	
Crosses x Doses	N.S.				N.S.			

Means followed by different letters (capital and small) differ significantly as determined by DMR Test.

N.S. = Non-significant

Cross-1 = NIAB-78 X USA-35

Cross-2 = NIAB-78 X USA-37

Cross-3 = NIAB-78 X RQB-62

Table 3. Matured plants, (%age), phenotypic changes (% age) and fertility changes (% age) in control and M₁ population.

Treat- ments	Seeds planted	Matured plants (% age)			Phenotypic changes (% age)			Fertility changes (% age)					
		Cross-1	Cross-2	Cross-3	Mean	Cross-1	Cross-2	Cross-3	Mean	Cross-1	Cross-2	Cross-3	Mean
To	220	68.2	68.9	70.0	69.0 B	0.0	0.0	0.0 D	0.0	0.0	0.0	0.0	0.0 D
T1	440	72.8	74.9	76.0	74.6 A	35.1	37.0	34.0	35.4 C	18.3	19.9	19.9	19.4 C
T2	320	68.9	71.1	65.9	68.6 B	59.6	58.4	69.9	62.6 B	25.7	27.4	26.6	26.6 B
T3	150	24.8	26.8	24.0	25.2 C	97.3	95.4	94.3	95.7 A	94.6	97.7	97.1	96.5 A
T4	10	0.0	0.0	0.0	0.0 D	0.0	0.0	0.0	0.0 D	0.0	0.0	0.0	0.0 D
Mean		46.9 A	48.3 A	47.2 A		38.4 A	33.2 A	39.6 A		27.7 B	29.0 A	28.7 AB	
Coefficient of variation			3.26%				8.3%						2.49%

Means followed by different letters differ significantly as determined by DMR Test.

Cross-1 = NIAB-78 X USA-35 To = 0 kR T3 = 2.0 kR

Cross-2 = NIAB-78 X USA-37 T1 = 0.5 kR T4 = 5.0 kR

Cross-3 = NIAB-78 X ROB-62 T2 = 1.0 kR

Discussion

The results indicated that comparatively low doses of gamma radiation should be used to avoid extensive damage to the embryo and seed. Largest number of bolls and seed (Table 1 and Table 2) in T_1 treatments possibly may be due to the stimulation of pollen tube growth resulting in an increase in fertilization (Seibold *et al.*, 1979; Aslam & Stelly, 1992). With the increase in the radiation doses to pollen, the boll set and number of seeds per treatment decreased (Pate & Duncan 1963; Krishnaswami & Kothandaraman, 1976), indicating that pollen fails to function at higher doses. The damage to the pollen nuclei may be the cause of embryo abortion. The elongation of pollen tube is a physiological process and takes place independently of the pollen nuclei. Even high doses of ionizing radiations which damage the pollen nuclei do not inhibit pollen germination (Brewbaker & Emery 1962). Most of the gross phenotypic and fertility changes in the M_1 generation are probably the result of chromosomal aberrations induced by radiations. On the basis of the results obtained it may be concluded that pollen irradiated at 0.5 to 1.0 kR of gamma rays before cross-pollinations is suitable for inducing useful genetic variability in cotton (Pate & Duncan, 1963; Krishnaswami & Kothandaraman, 1976). There was no difference in varietal sensitivity to radiation in *G.hirsutum*. This is in contrast to the differential varietal response observed in the same species when the seeds were irradiated (Khan *et al.*, 1982). Pollen irradiation in cotton to induce genetic changes with gamma-rays (Pate & Duncan, 1963) and x-rays (Jagathesan & Puri, 1965) enhanced genetic variability in the progeny derived from the pollen treated with radiation. The ease with which the cotton flowers can be emasculated and pollinated is a factor which also encourages the adoption of this technique for cotton improvement.

Acknowledgements

The authors are highly indebted to Dr. M. Afsar Awan, Head, Mutation Breeding Division NIAB, Faisalabad for critically reviewing the manuscript.

References

- Al-Didi, M.A. 1965. Development of new Egyptian cotton strains by seed irradiation. The use of Induced Mutations in Plant Breeding, *Radiat. Bot. Suppl.*, 5: 579-583.
- Aslam, M. and D.M. Stelly. 1992. Attempted egg-transformation by pollen irradiation in the cotton genus, *Gossypium*. *Proc. Int. Symp. on "New genetical approaches for crop improvement*. 15-20 Feb. Karachi, Pakistan.
- Brewbaker, J.L. and G.C. Emery. 1962. Pollen radiobotany. *Radiat. Bot.*, 1: 101-154.
- Carnelius, T.J. 1973. A new cotton variety MCU-7 by X-ray irradiation. *Mutat. Breed. Newsl.*, 2.
- Iqbal, R.M.S., M.B. Chaudhry, M. Aslam and A.A. Bandesha. 1991. Economic and agricultural impact of mutation breeding in cotton in Pakistan- a review. *Plant Mutation Breeding for Crop Improvement*, IAEA-SM-311/7, 1: 187-201.
- Jagathesan, D. and R.P. Puri. 1965. Pollen irradiation in cotton. *Ind. Cotton J.*, 19: 18-21.
- Jinks, J.L., P.D.S. Caligari and N.R. Ingram. 1981. Gene transfer in *Nicotiana rustica* using irradiated

- pollen. *Nature*, 291: 586-588.
- Khan, M.S.I., M. B. Chaudhry, A. A. Bandesha and M. Aslam. 1982. Radiosensitivity studies in cotton. *The Nucleus*, 19: 25-29.
- Krishnaswami, R. and R.Kothandaraman. 1976. Response of cotton pollen to gamma-irradiation. *Ind. J. Genet. Plant Breed.*, 36: 16-19.
- Micke, A. and B. Donini. 1982. Use of induced mutations in improvement of seed propagated crops. Induced Variability in Plant Breeding. *Proc. Int. Symp. Wageningen*, 1981, Wageningen Centre for Agricultural Publishing and Documentations: 2-9.
- Micke, A., B.Donini and M.Maluszynski. 1987. Induced mutations for crop improvement- a review. *Trop. Agric. (Trinidad)*, 64: 259-278.
- Pate, J.B. and E.N. Duncan. 1963. Mutations in cotton induced by gamma irradiated pollen. *Crop Sci.*, 3: 136-138.
- Pandey K.K. 1978. Gametic gene transfer in *Nicotiana* by means of irradiated pollen. *Genetica*, 49,1: 53-69.
- Seibold, H.W., L. Zelles and D.E.W.Ernst.1979. Tube growth stimulation of pine pollen by low doses of irradiation. Dose rate reproducibility and comparison between UV-light and ionizing rays. *Radiat. Environ. Biophys.*, 16. 107-116.
- Steel, R.G.D. and J.H. Torrie. 1980. *Principles and procedures of statistics*. McGraw Hill Book Co., Inc., New York, USA.

(Received for Publication 17 April 1993)