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## Evolution of High Yielding, Early Maturing and CLCuV Resistant Mutant of Cotton NIAB-98, Through the Use of Pollen Irradiation Approach

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**Abstract:** The locally well adapted cotton variety NIAB-78 was crossed with an exotic line REBA-288 with male parent pollen irradiated at 10 Gy of gamma rays before cross-pollinations to develop  $M_0$  seed. The  $M_2$  population was grown from  $M_1$  seed and different desirable mutants having higher yield, early maturity, short internode, resistant to CLCuV disease etc. were selected. These mutants were evaluated for yield potential and other economic traits in different segregated generations till uniformity was achieved. Of these the mutant PIM-76-8, later named as NIAB-98 was finally selected. The mutant NIAB-98 has a medium sized plant with suitable plant type and better leaf foliage. It is moderately hairy, early maturing, heat tolerant, has high yield potential and has acceptable fibre characteristics. At NIAB on the average, NIAB-98 gave 35.1 % higher yield than CIM-443, 9.1 % higher yield than NIAB-Karishma, 35.8 % higher yield than CIM-482 respectively during the years 1998-99 to 2001-2. At farmers fields (3 years average) it gave 21.0, 16.1, 9.2 29.9 and 16.9% higher yield than CIM-443, NIAB-Karishma, CIM-482, CIM-446 and FH-900 was respectively during the years 1999-2002. In NCVT and DCR trials conducted by PCCC, Karachi and Director Cotton Research Institute Faisalabad, it showed wider adaptability right from Faisalabad down towards Sindh by ranking 4th in the NCVT -2000-2001 and in the DCR trials ranked 7th during 2000-2001 and 2nd during 2001-2002 respectively in the said trials. Moreover it showed early maturity, heat tolerance, relatively better tolerance to bollworms, particularly pink bollworm. It can escape the peak attack of the same due to its early maturity. It has higher oil and protein contents i.e. 24 and 28 % respectively than leading/standard cotton varieties (CIM-433, CIM-446, CIM-482, CIM-473 and FH-900).

**Key words:** Evolution, pollen irradiation, cotton mutant NIAB-98, male pollen, high yield, gamma rays, generations, CLCuV disease

### Introduction

The success of all conventional cotton breeding approaches is highly correlated with the genetic variability present within the existing germplasm. However if the desired trait is not present or/and linked with other undesirable traits in the existing germplasm, then the cross breeding may not be worthwhile. In such cases recombination of genes is to be sought out to achieve the desired objectives. Recombination process plays a major role to induce genetic changes and the recombination of linked genes is brought about by crossing over. Generally in eukaryotic cells radiation treatments are known to enhance crossing over in proximal region adjacent to the centromere. Thus irradiation of  $F_1$  plants especially during premeiotic stages is further known to enhance crossing over in proximal region adjacent to the centromere resulting in further enhancing the genetic variability in  $F_2$  population. Increased variability in  $F_2M_2$  for quantitative traits has been reported in rice (Jalil Miah and Yamaguchi, 1965). Moreover radiation as well as several chemicals are reported to increase somatic recombinations (Vig, 1973). Exposure of seed to ionizing radiation's has resulted in creating genetic variability in different crop species and many plant breeding programmes have shown the feasibility of radiation plus selection as a direct method of varietal improvement (Carnelius, 1973; Micke *et al.*, 1987; Iqbal *et al.*, 1991; Iqbal *et al.*, 1994). Irradiation of male parent pollen before cross-pollinations resulted in the induction of mutations in cotton (Pate and Duncan, 1963; Krishnaswami and Kothandaraman, 1976). The studies carried out by Aslam and

Stelly (1994) and Aslam *et al.* (1994) and Aslam (2002) have shown that treatment of pollen with low doses of gamma rays (5 to 20 Gy) before cross-pollinations are suitable to induce useful genetic variability in cotton. The present research studies were aimed at to create genetic variability through crosses with irradiated male parent pollen, selecting the desirable recombinants from the segregating populations and their evaluation for high yield potential and wider adaptability in the cotton growing areas of Punjab for confirmation.

### Materials and Methods

The well adapted and higher yielding local cotton variety NIAB-78 was crossed with an exotic line REBA-288 using irradiated male parent pollen at 10 Gy of gamma rays before cross-pollinations during the year, 1993-94. At maturity the seed cotton was collected from the bolls obtained from successful crosses and ginned to produce  $M_0$  seed.  $M_1$  population was grown from  $M_0$  seed at a spacing of 30 and 75 cm from plant to plant and row to row respectively. At maturity the seed cotton was collected from  $M_1$  population and instead of pooling one locule per boll from all the  $M_1$  plants together, we picked seed cotton one locule from each boll of each  $M_1$  plant separately. The  $M_2$  population comprising of about more than three hundred individual plants was studied and selection for the desirable mutants/recombinants was carried out. The selections from the  $M_2$  was based upon, early maturity, better plant type, higher yield and better yield components etc., along with resistance to CLCuV disease. Of these, 12 promising mutants were grown in  $M_3$  generation in replicated plant progeny test (RPT). The size of the individual plot was  $0.75 \times 10 \text{ m}^2$ . The breeding behaviour of these progenies was studied in  $M_3$  generation and a higher yielding progeny, PIM-76-8 was selected. The same was studied in plant progeny rows in  $M_4$  generation to confirm its higher yield potential and to see its breeding behavior/uniformity. Finally the progeny PIM-76-8 was selected from  $M_5$  and bulked for evaluation under the name of NIAB-98. During the years i.e., 1999-2002, various trials (zonal yield trials, NCVT and DCR etc., were conducted at NIAB, at farmers fields etc., for confirmation of the high yield potential and wider adaptability. Moreover various other related studies i.e., on earliness, heat tolerance, reaction towards insects and diseases particularly CLCuV disease were carried out.

### Results and Discussion

The  $M_1$  generation results revealed that the plants were faster in growth and had hybrid vigour for various traits. The  $M_1$  generation plants showed resistance to CLCuV disease under severe natural disease epidemic (S-12, a highly susceptible variety had 100 % CLCuV disease infestation). It was noted that the  $M_2$  generation plant progenies were generally of varied nature and some of the individual plants possessed desirable combination of certain economic traits alongwith resistance against CLCuV disease under high disease infestation. The boll weight and yield of the promising mutants selected from  $M_2$  population ranged from 3.5-5.0 g and 211-396 g respectively. Then the mutant was evaluated in the succeeding segregating generations. The results indicated that the mutant progeny, PIM-76-8 gave 22.5% higher average yield than the prevalent standard cotton variety, CIM-443. The morphological studies carried out indicated that the plant of NIAB-98 is moderately hairy, semi-compact sympodial type with fruit bearing monopodia. It has desirable leaf foliage and medium plant stature. The length of the sympodial and monopodial and internodes of the main stem are shorter than those of the

**Aslam: Evolution, pollen irradiation, cotton mutant NIAB-98, male pollen, high yield**

**Table 1: Morphological characteristics and fruiting pattern of mutant NIAB-98 compared with other varieties of cotton**

Varieties	Main stem height (cm)	Boll weight (g)	Internodal length (cm)	Monopodia (No.)	Monopodia length (cm)	Sympodia length (cm)	Hairiness (Grade)	Abscission (%)	Days to Maturity	Total fruiting points/p	Fruit intact/P
NIAB-78	110-115	3.0B	3.0D	0-1	65D	40-45	5-6	35-40	140-150	200A	115A
NIAB-98	130-135	3.5A	3.5C	0-3	55C	25-40	4-5	30-35	145-150	180B	120A
CIM-443	130-135	2.9B	4.0B	0-3	50C	25-30	5-6	45-50	140-150	160C	75B
REBA-288	150-160	3.8A	5.0A	0-5	75A	40-45	4-5	55-65	160-170	175B	55C

**Table 2: Confirmation of CLCuV resistance in NIAB-98, through artificial grafting in NCVT (2000-2001) by CRI Multan/reported by PCCC, Karachi**

Varieties	CLCuV Reaction*	Number of days taken to appear the Symptoms (After grafting) (%age)	Grade intensity	Incidence under field conditions
V 1 SLH-244	-	Disease did not appear	0 **	0.0
V 2 NIAB-98	-	Disease did not appear	0	0.0
V 3 CIM-448	-	Disease did not appear	0	0.0
V 4 MNH-633	-	Disease did not appear	0	0.0
V 5 BH-124	+	60	1	3.3
V 6 RH-500	-	Disease did not appear	0	0.0
V 7 CIM-473	-	Disease did not appear	0	0.0
V 8 CRIS-467	+	50	1	3.5
V 9 CRIS-134	+++	15	6	88.1
V 10 DNH-49	+	15	6	97.0
V 11 CIM-482	-	Disease did not appear	0	0.0
V 12 BH-125	-	Disease did not appear	0	0.0
V 13 NIAB-999	+	50	6	5.9
V 14 MNH-356	+	50	6	6.4
V 15 CRIS-82	+++	15	1	97.1
V 16 FH-900	-	Disease did not appear	0	0.0
V 17 PPNH-945	-	Disease did not appear	0	0.0
V 17 SAEC-78389	+++	50	6	100.0
V 18 BH-118	-	Disease did not appear	0	0.0
S-12	+++	15	6	100.0

\*- = No symptoms; + = Mild; ++ = Medium; +++ = Severe; \*\* 0 = Complete absence of symptoms; 1 = Few small scattered vein thickening; 2 = small scattered vein thickening; 3 = Vein thickening involving small vein thickening; 4 = Large groups of vein involved; 5 = All vein involved; 6 = All vein involved and severe curling

**Table 3: Performance of mutant NIAB-98 in yield trials in comparison to standard cotton varieties at NIAB (1998-2002)**

Mut./ Var.	CLCuV disease (grade)* reaction	Yield/ha (Kg)	% Increase of NIAB-98 over
Average yield (3 trial's 98-99)			
NIAB-98	(0)	3420.1	-
CIM-443	(0)	2590.3	32.0
Average yield (3 trial's 99-2000)			
NIAB-98	(0)	5310.2	-
CIM-443	(0)	3606.9	47.2
N-Karishma	(0)	3606.9	47.2
Average yield (3 trial's 2000-1)			
NIAB-98	(0)	3912.2	-
CIM-482	(0)	3075.3	27.2
Average yield (3 trial's 2001-2)			
NIAB-98	(0)	4105.3	-
CIM-482	(0)	3182.9	28.9
Overall average			
NIAB-98	(0)	4186.9	-
CIM-443	(0)	3098.6	35.1
N-Karishma	(0)	3836.7	9.1
CIM-482	(0)	3129.1	33.8

\*Rating Scale 0-5, 0 = No symptoms, 5 = Highly susceptible

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**Table 4: Summarized (average) yield performance (Kg/ha) of mutant NIAB-98 at farmers fields during the years 1999-2002**

Year	NIAB-98	CIM-443	NIAB-K	CIM-482	CIM-446	FH-900	CIM-473
1999-2000	3096.4	2439.2	2414.3	3600.2	2255.5	2742.0	-
Locations	(30)	(12)	(17)	(2)	(5)	(6)	
2000-2001	2990.6	-	2736.6	2431.0	2212.0	2492.4	2764.5
Locations	(59)		(21)	(20)	(23)	(21)	(30)
2001-2002	2770.5	-	2475.1	2079.5	2353.3	2344.1	2547.2
Locations	(55)		(11)	(15)	(13)	(24)	(17)
Average	2952.5	2439.2	2542.0	2703.5	2273.6	2526.2	2655.8
Locations	(144)	(12)	(49)	(37)	(41)	(51)	(47)
%Increase over	-	21.0	16.1	9.2	29.9	16.9	11.2

**Table 5: Yield results of NCVT-2001, DCR-2000-2000 and DCR-2000-2001**

NCVT-2000-2002		DCR-2000-2001		DCR-2001-2000	
Varieties	Overall average	Varieties	Overall average	Varieties	Overall average
V1 SLH-244	1970.3	VH-59	2271	MNH-536	1701
V2 NIAB-98	2404.8V	MNH-564	1725	SLH-257	2083
V3 CIM-448	2054.5	FH-900	2201	CIM-499	2181
V4 MNH-633	2127.0	NIAB-999	2498-II	NIAB-999	2084-V
V5 BH-124	2184.5	RH-500	2466-III	FH-930	2001
V6 RH-500	2121.5	CIM-473	2537-I	BH-147	2193-IV
V7 CIM-473	2127	SLH-247	2069	ALSEEMI	2283-I
V8 CRIS-467	2358.3	493/97	2069	VH-141	2006
V9 CRIS-134	26.23.3I	FH-901	2455	FH-901	2095
V10 DNH-49	2450.3IV	NIAB-98	2456-IV	MNH-633	2095
V11 CIM-482	1980.3	BH-147	2451-V	FH-900	2058
V12 BH-125	2471.8II	CIM-448	2306	MNH-64.2	1826
V13 NIAB-999	2477.0	BH-121	2249	NIAB-98	2246-II
V14 MNH-356	2082.8	SLH-242	2204	CIM-473	2092
V15 CRIS-82	2518II	BH-125	2498-II	CIM-482	2008
V16 FH-900	2153.8	K-99	2289	K-99	2085
V17 PNH-945	-	VH-137	2369	RH-510	1897
V18 BH-118	2249.8	BH-124	2262	BH-121	2135
V19 -	-	MNH-536	2143	NIAB-228	2158-VI
V20 -	-	-	-	FH-1000	2213-III

**Table 6: Effect of high temperature on fruit formation in NIAB-98 and other leading cotton varieties during 2001-2.**

Varieties	Bolls formed*(No.)	Flowers *(No.)	Squares *(No.)	Total fruiting points
NIAB-98	43.8A	6.4A	14.0AB	64.2
CIM-482	34.2B	4.4C	11.6B	50.2
CIM-473	17.8C	5.0B	17.2A	40.0

\* Average fruit formation from July - August 22, 2001.

**Table 7: Earliness studies on NIAB-98 and other standard cotton varieties during the year, 1999 and 2000 ( average)**

Observations	Duration in days			
	NIAB-98	CIM-443	NIAB-Karishma	CIM-482
First flower appearance after sowing	3B	39B	48A	45A
Boll maturity period after the initiation of flower	45C	44C	51A	48B
Total Days	83	83	99	93

parents and prevalent commercial varieties. Compared with the tall plants forming a canopy at the top, this medium plant (4-5.5 feet) is better suited for better photosynthetic activity and better yields. This plant type makes the agricultural operations such as pesticides spray, picking, inter-culture, etc., also easier (Table 1). The morphological characters of NIAB-98 make its growing period shorter with faster squaring quality and it matures in 145-150 days with high fruiting load to give high yield. The seeds of NIAB-98 are bold dull white and fuzzy with greenish tinge.

The results revealed that NIAB-98 was resistance to CLCuV under high inoculation in the field. The CLCuV resistance was further confirmed through artificial grafting. The results revealed that NIAB-98, not only showed resistance to CLCuV disease under field conditions, but also showed no disease symptoms even though the disease was transferred through artificial grafting (Table 2). Various trials were conducted at NIAB and on Farmers

Fields and coordinated varietal trials organized by PCCC, Karachi, and Director Cotton Research Institute, Faisalabad. The results revealed that at NIAB, on the average the NIAB -98 gave 32.0 % higher yield than CIM-443 during 1998-99 and during 1999-2000 it gave 47.2 and 37.6 % higher yield than CIM-443 and NIAB-Karishma respectively (Table 3). Whereas the increase in yield of NIAB-98 over the latest standard cotton variety CIM-482 was 27.2 % during 2000-2001 and 28.9 during 2001-2002 respectively. The trials conducted at farmer's fields; revealed that on the average ( average of 3 years) the NIAB-98 gave 21.0,16.1, 9.2, 29.9,16.9 and 11.2% higher yield over the standard varieties i.e. CIM-443, NIAB -Karishma CIM-482, CIM-446 and FH-900 respectively during the year 1999-2002 (Table 4). The NIAB-98 also had shown wider adaptability by ranking 4th in NCVT 2000-2001 and 6th and 2nd in DCR trials, 2000-2001 and 2001-2002, respectively as compared with the entire standard leading varieties (Table 5).

Aslam: Evolution, pollen irradiation, cotton mutant NIAB-98, male pollen, high yield

Table 8: Mean population development of sucking pests and bollworms in different cotton varieties (2000-2001)

Varieties	Source	Jassid	Whitefly	Bollworms
V 1 SLH-244	CRS Sahiwal	3.4E	5.8E	16.6
V 2 NIAB-98	NIAB, Faisalabad	3.9C	6.9C	15.5K
V 3 CIM-448	Standard	3.6CD	5.4FG	15.8I
V 4 MNH-633	CRS Multan	3.9A	4.8O	17.3H
V 5 BH-124	CRS Bhawalpur	2.8F	4.0I	20.2C
V 6 RH-500	CRI, Rahim Yar Khan	3.9A	5.9E	14.8M
V 7 CIM-473	CCRI, Multan	3.4E	7.8B	19.3E
V 8 CRIS-467	CCRI, Sakrand	3.5DE	5.3G	15.1L
V 9 CRIS-134	CCRI, Sakrand	3.7BC	6.7D	18.1G
V 10 DNH-49	CRS, D. I. Khan	3.4E	3.9I	22.3A
V 11 CIM-482	CCRI, Multan	3.6CD	8.5A	19.5D
V 12 BH-125	CRS Bhawalpur	3.6CD	5.3G	18.8F
V 13 NIAB-999	NIAB, Faisalabad	3.6CD	5.5F	17.3H
V 14 MNH-356	CRS Multan	3.8AB	5.0H	21.9B
V 15 CRIS-82	CCRI, Sakrand	3.9A	5.1H	15.8I
V 16 FH-900	CRI, Faisalabad	3.6BC	6.9C	16.5I
V 17 PNH-945	CRI, Faisalabad	3.5DE	5.3G	13.3N
V 18 BH-118	CRS Bhawalpur	3.6CD	5.0H	15.8I

Table 9: Population of sucking insect pest's on various genotypes of cotton (Per leaf population) by Entomologist AARI, Faisalabad, during 2001-02

Genotypes	Jassid Nymph	Jassid Adult	Whitefly Nymph	Whitefly Adult	Thrips
BH-124	0.128A	0.668C	1.045AB	0.200A	0.297BC
BH-121	0.166A	0.958AB	1.262A	0.355A	0.411BC
BH-147	0.138A	1.058A	1.014AB	0.201A	0.552ABC
BH-125	0.145A	0.823ABC	0.752BC	0.110A	0.635AB
SLH-257	0.048A	0.637C	1.199A	0.352A	0.895A
SLH-224	0.086AB	0.655C	1.230A	0.143A	0.845A
NIAB-98	0.043B	0.724BC	0.520C	0.285A	0.197C

Mean sharing similar letters are not significantly different at P=0.05.

Table 10: Summarized-quality characters of NIAB 98, compared with other cotton varieties determined by NIAB and other fibre testing Laboratories

Year/trial	NIAB-98					CIM-443					NIAB-K				
	G.O.T.	SL	FF	FS	MI	G.O.T.	SL	FF	FS	MI	G.O.T.	SL	FF	FS	MI
1998-99 M-VT	38.0	27.2	4.7	92.0	94.0	-	-	-	-	-	38.0	28.0	4.9	89.0	91.0
1999-00 VT(1-3)	37.0	27.6	4.8	90.0	92.0	38.0	27.2	5.2	87.1	91.2	36.6	26.5	5.1	88.9	90.5
2000-01 VT(1-3)	37.0	27.6	4.6	86.1	91.2	38.5	27.1	4.9	91.3	78.5	36.9	26.3	4.6	89.6	86.0
2001-02 VT(1-3)	39.2	27.4	4.8	91.5	86.2	37.8	28.4	4.9	75.6	79.0	37.5	26.1	4.7	87.1	87.0
2000-01-NCVT	38.4	27.0	4.9	88.4	89.3	-	-	-	-	-	-	-	-	-	-
2000-01-DCR	39.0	28.0	4.6	91.0	86.2	-	-	-	-	-	-	-	-	-	-
2001-02 NCVT	38.0	27.6	4.6	90.6	86.2	-	-	-	-	-	-	-	-	-	-
1999-2000 F. Field	38.0	27.5	5.0	92.0	98.0	-	27.7	5.1	-	98.1	-	28.1	5.3	-	97.1
2001-2002 "	37.5	27.0	4.6	47.9**	87.3	-	-	-	-	-	36.9	26.9	4.6	-	88.0
2001-02 S.E-NIAB.	36.5	26.6	4.8	-	88.6	35.9	24.7	5.6	-	94.2*	38.3	28.6	4.4	-	85.2-FH-1000
2001-02 U.A.F.	38.0	28.5	4.9	27.5+	48.5**	-	-	-	-	-	38.0	26.5	4.5	27.0+	78.5
2001-02 C.TM.	37.5	27.1	4.9	27.8+	84.5	-	-	-	-	-	39.0	27.9	5.0	26.3+	46.3**
Average	37.8	27.4	4.8	90.2	89.4	38.1	27.4	5.0	84.7	82.2	37.5	27.0	4.8	88.6	88.3
2000-01 S.E.	36.6	28.1	5.3	97.0	-	-	-	-	-	-	-	-	-	-	-
2001-02 S.E.	35.7	28.1	5.6	29.8+	-	-	-	-	-	-	-	-	-	-	-
2001-02 SE	39.0	27.3	5.3	25.6+	-	-	-	-	-	-	-	-	-	-	-
Overall average	37.7	27.5	4.9	27.5+	89.4	37.5	27.4	5.0	84.7	82.2	37.5	27.0	4.8	88.6	88.3
		UR = 48.2									UR = 46.3				26.6 g/Tex*

= MNH-552 + = Strength gm/Tex \*\* = Uniformity Ratio

S.E. = Spot Examination

Table 11: Oil and Protein contents (%) of different cotton varieties during 2001-2002

Variety	Oil contents (%)	Protein contents (%)
FH-900	20.2	27.5
NIAB-98*	24.2	28.0
CIM-473	20.1	27.3
CIM-446	21.4	26.7
NIAB-98**	19.8	27.2
CIM-443	19.2	27.1
CIM-482	20.3	27.1

\*NIAB Faisalabad; \*\* PSC Farm, Khanewal

The results on earliness and physiological attributes of NIAB-98, showed that the flowering started earlier in NIAB-98 and is better tolerant to high temperature as compared to other varieties (Table 6, 7). Moreover it is also apparent that NIAB-98 had shorter boll maturity period thereby confirming the earliness of NIAB-98. The results of studies carried out on relative development of sucking pests and bollworms complex on different cotton varieties during 2000-2001 indicated that NIAB-98 was comparable or some time better with respect to sucking pest's complex development than other varieties (Table 8, 9). Whereas it had relatively less infestation of bollworms and due to its early maturity it can escape the peak attack period of pink bollworm and fewer number larvae enter into diapause thus reducing the carry-over source

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for infestation for the next year. The fibre characteristics of NIAB-98 were tested at NIAB. The results indicated that the all value have been comparable and acceptable (Table 10). NIAB-98 has G.O.T. of 37.7%, staple length of 27.5mm, fineness of 4.9  $\mu\text{g}/\text{in}$ , strength of 93.5 TPPSI, fibre maturity 89.4% and uniformity ratio of 48.2%. The results of the studies carried out on oil and protein contents of NIAB-98 revealed that NIAB-98 had higher percentage of oil contents i.e., 24% and also protein contents i.e. 28% (Table 11) as compared to prevalent standard cotton varieties (CIM-433, CIM-446, CIM-482, CIM-473 and FH-900).

The pollen irradiation approach seem to be better than seed irradiation, since in order to create genetic variability through seed irradiation, the whole genome is to be irradiated, which ultimately disturb the whole genetic makeup of the exposed individual. Consequently the most of the changes occurring in the population are the somatic/non-heritable changes. Therefore large  $M_2$  population may be more than 12,000 individual plants is required, to select desirable mutants (Iqbal *et al.*, 1994). Since the irradiated pollen is a germ cell and after fertilization only half of the genome of the developing zygote/embryo, receives the irradiation, hence the occurrence of major changes is minimized as observed in case of seed irradiation. The results confirmed that the pollen irradiation is a valuable technique, which can be employed to improve crop plants most effectively. Moreover incase of seed irradiation usually from each  $M_1$  plant the seed cotton from each locule per boll is collected and then pooled to have  $M_1$  seed to grow  $M_2$  population and consequently large population has to be screened for selecting the desired genotype. But through the use of pollen irradiation technique, each  $M_1$  plant has to be grown separately as plant progeny rows to develop  $M_2$  population, which facilitate to carry out selection. Since most of the progenies carried micro- mutations/point mutation due to optimal radiation dosages applied to pollen before fertilization and no major abnormalities were noticed which may help to achieve uniformity earlier as compared to seed irradiation. Moreover the male gamete is to be irradiated at low doses of gamma rays before fertilization and therefore more recombinations are brought about due to enhanced chiasmata formation/crossing over during meiotic stages of cell divisions. The results reported above have clearly illustrated that from a very small  $M_2$  population even less than 1000 plants, higher rate of mutations/recombinations was achieved through pollen irradiation. Therefore the results obtained clearly confirmed the earlier findings (Jalil and Yamaguchi, 1965, Vig, 1973, Wang, 1990). Moreover, the method of gamete treatment was found easier to apply than that of zygote/seed treatment.

Irradiation of male parent pollen before cross-pollinations resulted

in the induction of mutations in cotton (Pate and Duncan, 1963; Krishnaswami and Kothandaraman, 1976). These results are in accordance with the earlier findings (Aslam and Stelly, 1994; Aslam *et al.*, 1994; Aslam, 2000), that the treatment of pollen with of low doses of gamma rays (5 to 20 Gy) before cross-pollinations are suitable to induce useful genetic variability in cotton, *G. hirsutum* L.

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