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Induction of Mutations Through Crosses with Gamma Irradiated Pollen in Cotton

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ABSTRACT

Cotton pollens were exposed to gamma irradiation to create genetic variability for the selection of desirable mutants from the segregating generations before cross-pollinations. The suitable dose for application to cotton pollen before cross-pollinations to induce desirable mutations was from 5-10 Gy of gamma rays. During the year 1999-2000, different crosses (VH-137 x CIM-448, CIM-435 x DNH-49, BH-36 x BH-36, CIM-1100 x PIM-77-2, DNH-49 x PIM-98) were made using gamma irradiated male parent pollen. The M_1 seed was developed and M_2 populations were grown for selection. The desirable mutants were selected from M_2 population. The boll weight and yield of the selected mutants ranged from 3.3 to 5.0 g and 250-350 g/plant, respectively. The selected mutants possessed desirable fibre quality traits. During the year 2002-2003, the elite mutants were studied in M_3 segregating generation. These mutants depicted higher yield potential, better boll size, early maturity etc., along with resistance to CLCuV disease. Elite advanced mutants developed through pollen irradiation approach were also evaluated under the project during the year 2001-2003. The details of these results have been described and discussed in this paper.

Key Words: Mutations; Crosses; Gamma radiation; Cotton pollen; *Gossypium hirsutum* L.

INTRODUCTION

Upland cotton being the most important cash crop of Pakistan, occupies the second largest area among all of the crops. It is cultivated over an area of 3125,000 ha (ICAC, 2002-03). Cotton lint besides earning huge amount of foreign exchange through export also provides raw material to the local textile industry. In addition to the lint, cottonseed accounts for 71% of the domestic oil production (GOP, 2001-2002) and feed (seed cake) for dairy animals. Overall the living of millions of people in Pakistan is linked with cotton cultivation, ginning, oil industries, trade and spinning processes.

During the past decade, the Cotton Leaf Curl Virus (CLCuV) disease emerged as the most important disease of cotton in Pakistan. This viral disease was first noticed near Multan during 1967 (Hussain & Ali, 1975). It reached economic importance in 1987-88 and became epidemic during 1993-94. Due to which the cotton production of 12.8 million bales in 1991-92, dropped to 8.0 million bales of lint in 1993-94. Measures such as the control of insect vector and crop rotation may help to minimize losses, but the resistant cotton varieties must be developed to overcome this epidemic. The breeders of the country made extensive efforts to develop CLCuV resistant cotton varieties through conventional breeding methods. However the limitations were the non-availability of the resistant native germplasm. The approaches like; the exposure of seed to ionizing radiations (Carnelius, 1973; Micke *et al*., 1987; Iqbal *et al.*, 1991, 1994) and the treatment of pollen with low doses of gamma rays before cross-pollinations (Aslam & Stelly, 1994; Aslam, *et al.*, 1994; Aslam, 2000, 2002), resulted in creating genetic variability in different crop species. Moreover besides radiations, several chemicals are reported to increase somatic recombinations (Vig, 1973). The favourable mutation frequency was also higher increase of pollen irradiation than that of seed treatment (Wang, 1990). Moreover, the method of gametes treatment was easier to apply than that of zygote/seed treatment. The increased variability in F_2/ M_2 for quantitative traits has been reported in rice (Miah & Yamaguchi, 1965). The irradiation of pollen before cross-pollination resulted in the induction of mutations in cotton (Pate & Duncan, 1963; Krishnaswami & Kothandaraman, 1976). Under the Pak-Kazakh project the pollen irradiation technique was used to overcome the problem of cotton leaf curl virus disease by creating the genetic variability and selecting the desirable mutants in cotton.

MATERIALS AND METHODS

The work under Pak-Kazakh project, "Development of leaf curl virus tolerant varieties of cotton" was initiated during the year 1998-99. The Fifty-three different indigenous genotypes of cotton were screened for CLCuV disease resistance and for other economic traits. These were found not to be possessing good combination of desirable traits along with resistance to CLCuV disease. It was noted that the genotypes carrying some identified genes of economic value can be manipulated and utilized for the

synthesis of the requisite genotypes which would better adopt to the varying climatic conditions of the country. Out of the selected genotypes, VH-137, CIM-435, BH-36, CIM-1100 and DNH-49 were resistant to CLCuV disease. Therefore, these were chosen for the improvement of specific characters i.e., yield, earliness etc. While, other selected genotypes viz., PIM-98, PIM-77-2, PIM-76-2, CIM-448 and CIM-1098 possessing some desirable traits but susceptible to CLCuV disease were utilized as valuable gene pool in the cross breeding programme.

During the year 1999-2000, the following cross combinations were made with male parent pollen irradiated at 5 Gy, 10 Gy, and 20 Gy of gamma rays before cross-pollinations.

i) VH-137 X PIM-98
ii) CIM-435 X DNH-49
iii) BH-36 X BH-36 (pollinations with irradiated self-male pollen)
iv) CIM-1100 X PIM-77
v) DNH-49XPIM-98

At maturity the seed cotton from the crossed bolls of the cross-combinations was harvested/ginned and M_0 seed was developed. The M_1 population was grown from M_0 seed in greenhouse to carry out preliminary studies and in the field with spacing of 30 cm and 75 cm from plant to plant and row to row respectively. At maturity the seed cotton from M_1 population was collected, ginned and M_1 generation seed was developed. The M_2 population was grown from M_1 seed in the field with spacing of 30 cm and 75 cm from plant to plant and row to row respectively. The M_1 and M_2 populations were exposed to CLCuV disease under natural high disease infestation during the consecutive years, using spreader rows of a highly susceptible cultivar S-12 to encourage uniform inoculation. The highly susceptible cultivar S-12 received 100% disease infestation and this disease intensity was measured as described by Siddig (1968). From the M_2 population thirty eight mutants were selected on the basis of better boll weight, good opening, higher yield along with resistance to CLCuV disease etc. Out of these nineteen promising mutants were grown in plant progeny rows in M_3 generation to study their uniformity and breeding behavior. The size of the individual plot was 0.75m x 10m. From M_3 generation, the higher yielding mutants/ progenies were selected for further evaluation.

Moreover, to enhance the spectrum of genetic variability, the following fresh crosses using irradiated pollen were also made during the year 2000-2001 and 2001-2002, respectively.

Year 2000-2001	Year 2001-2002
DNH-49 x PIM-98	VH-137 x K-599
CIM-1100 x PIM-77-2	K-599 x CIM-1100
CIM-435 x DNH-49	DNH-49 x CIM-443
VH-137 x PIM-77-2	CIM-443 x L-31
BH-36 x BH-36	K-599 x VH137
CIM-435 x K-599	DNH-49 x K-599

The crossed seed obtained from the above mentioned cross-combinations were studied in different early generations accordingly. Moreover, the advanced mutants i.e., NIAB-98, NIAB-829, NIAB-824 and, NIAB-869, NIAB-785, NIAB-846, developed before the start of the project through pollen irradiation were also evaluated for yield potential and other economic traits under the project during the year 2001-2003.

RESULTS AND DISCUSSION

The preliminary studies carried out on number of bolls and seed produced for each treatment in various cross-combinations (VH-137 x PIM-98, CIM-435 x DNH-49, BH-36 x BH-36, CIM-1100 x PIM-77-2, DNH-49 x PIM-98) with irradiated pollen indicated that the boll setting and crossed seed production in various treatments differed significantly (Table I & II). Largest number of bolls and number of seeds were obtained from crosses made from 5 Gy of gamma rays treatment. However, the reduced boll setting and number of seeds from all the crosses were obtained from 10 Gy and 20 Gy of gamma ray treatments but this reduction was more significant in the boll setting and number of seeds produced in crosses from 20 Gy of gamma ray treatments. All the genotypes used in crossed showed similar response to gamma radiation applied to pollen before cross-pollinations. Whereas, there were significant differences in all the crosses in the boll setting, number of seeds produced and between the radiation doses applied to pollen. These results confirmed the earlier findings of Aslam *et al.* (1994).

The M_1 generation plants were more vigorous in growth and depicted hybrid vigour for various morphological traits and showed resistance against CLCuV disease under severe natural disease epidemic (S-12 highly susceptible variety had 100% CLCuV disease infestation). In M_2 generation, the plant progenies were generally of varied nature and certain individual plants possessed desirable combination of economic traits along with resistance against CLCuV disease under high disease infestation/epidemic (S-12 variety highly susceptible had 100% CLCuV disease infestation). These M_2 mutants possessed better boll weight and had higher yield as compared to latest standard cotton variety. The boll weight and yield per plant of the promising M_2 mutants ranged from 3.3 to 5.0 g and 118 to 350 g, respectively (Table III). Whereas, the boll weight and yield per plant of the latest standard cotton variety CIM-473 was 4.5 and 136 g, respectively. Moreover, the mutants were resistant to CLCuV disease, early maturing with desirable quality traits. The G.O.T., fibre length, fibre fineness and maturity index ranged from 34.0-40.8%, 27.0-30.2 mm, 3.9- 4.8 µg/in, and 80-88.3%, respectively (Table IV). The promising mutants of M_2 generation were studied as plant progeny rows to see their breeding behavior and to confirm higher yield potential in M_3 generation during 2002-2003. The yield of the

promising muatant progenies in M_3 generation ranged from 2898.0 to 4620.0 kg/ha as compared to 3691 kg/ha yield of CIM-473 (Table V). Moreover, certain M_3 generation progenies also revealed segregation for CLCuV disease resistance but most of the progenies preserved their better boll weight, higher yield potential and early maturity as compared to latest standard cotton variety CIM-473. Further evaluation of higher yielding, CLCuV disease resistant mutants will be continued to develop economically suitable varieties and germplasm of cotton.

From M_2 population from the crosses made in 2000-2001, about 75 different desirable mutants were selected. The boll weight and yield per plant of the selected M_2 mutants ranged upto 4.9 g and 305.0 g, respectively, during 2002-2003 (Table VI) while, the standard CIM-473 had boll weight of 4.3 g and yield of 150.0 g per plant, respectively. M_1 population raised from later crosses of made in 2001-2002 indicated hybrid vigour, resistance to CLCuV disease along with better yield than the latest standard cotton variety CIM-473 (Table VII).

Different advanced mutants i.e., NIAB-98, NIAB-829, NIAB-824 NIAB-869, NIAB-785, NIAB-846 were

Table I. Number of bolls produced in different crosses in cotton with irradiated pollen from 20 pollinations made for each cross

Irradiation Treatments	Number of bolls produced (No.)					
	Cross-1	Cross-2	Cross-3	Cross-4	Cross-5	Mean
0 Gy	8.6b	8.7b	8.4b	8.4b	8.1b	8.4B
5 Gy	10.8a	10.3a	10.5a	10.5a	10.3a	10.5A
10 Gy	7.2c	6.8c	7.1c	6.7c	6.9c	6.9C
20Gy	4.2d	4.3d	4.5d	4.2d	3.9d	4.2D
Mean	7.7A	7.5A	7.6A	7.4A	7.5A -	
Crosses x Doses	N.S.					

Cross-1= VH-137 x XPIM-98, Cross-2= CIM-435 x DNH-49, Cross-3= BH-36 x BH-36, Cross-4 = CIM-1100 x PIM-77-2, Cross-5= DNH-49 x PIM-98

Table II. Number of seeds produced in different crosses in cotton with irradiated pollen from 20 pollinations made for each cross

Treat.	Number of seeds produced (No.)					
	Cross-1	Cross-2	Cross-3	Cross-4	Cross-5	Mean
0 Gy	218.3b	221.2b	219.3b	221.2b	219.3b	219.9B
5 Gy	287.1a	289.2a	290.4a	292.5a	287.4a	289.3A
10 Gy	110.2c	111.8c	109.8c	106.4c	111.2c	109.9C
20Gy	51.2d	55.1d	52.4d	49.4d	53.4d	52.3D
Mean*	166.7A	169.3A	168.0A	167.4A	167.8A -	
Crosses x Doses	N.S.					

Cross-1= VH-137 x PIM-98, Cross-2= CIM-435 x DNH-49, Cross-3= BH-36 x BH-36, Cross-4 = CIM-1100 x PIM-77-2, Cross-5= DNH-49 x PIM-98; * Mean number of seeds for each cross

Table III. Characteristics (range) of promising M_2 mutants selected from crosses of 1999-2000 with irradiated pollen during 2001-2002

Crosses	Mutants Selected (No.)	CLCuV reaction	Plant height (cm)	Boll weight (g)	Yield/ plant (g)
VH-137 x PIM-98	8	R	150-190	3.6-4.6	143-233
CIM-435 x DNH-49	7	R	150-190	3.2-4.0	160-206
BH-36 x BH-36	5	R	84-160	3.5-4.6	134-148
CIM-1100 x PIM-77	14	R	132-150	3.3-4.7	118-350
DNH-49 x PIM-98	4	R	130-190	3.6-5.0	132-167
CIM-473	-	R	170	4.0	136
CIM-480	-	R	170	4.4	98.0
S-12	-	HS	160	4.0	105.0

R=Resistant to CLCuV; HS= highly susceptible to CLCuV

Table IV. Quality characteristics (range) of promising M_2 mutants selected from crosses of 1999-2000 with irradiated pollen during 2001-2002

Crosses	Mutants selected (No.)	G.O.T. (%)	Fibre length (mm)	Fibre fineness (µg/in)	Fibre maturity (%)
VH-137 X PIM-98	8	35.0-39.7	27.4-29.4	4.2-4.8	81.0-88.3
CIM-435 X DNH-49	7	35.6-40.6	27.0-30.2	4.0-4.6	82.0-86.8
BH-36 X BH-36	5	37.0-38.8	27.0-28.4	4.3-4.6	84.0-88.3
CIM-1100 X PIM-77	14	34.4-40.8	27.0-30.0	3.9-4.8	81.0-88.3
DNH-49XPIM-98	3	38.0-38.9	27.0-29.6	3.4-4.4	80.0-85.4
CIM-473	-	38	28.5	4.5	86.0

Table V. Yield performance of promising M_3 mutants/ progenies from Crosses of 1999-2000 with Irradiated pollen during 2002-2003

Crosses	M_3 Progenies (No.)	Mutant selected (No.)	CLCuV reaction	Yield/ha (kg)	% increase in yield over CIM-473
VH-137 X PIM-98	4	26	R	3723.7	22.10
CIM-435 X DNH-49	5	44	R	3768.5	123.48
BH-36 X BH-36	3	17	R	3583.0	17.40
CIM-1100 X PIM-77	5	23	R	3448.0	12.97
DNH-49XPIM-98	2	2	R	3000.4	-1.68
CIM-473	-	-	R	3051.9	-

Table VI. Characteristics of the promising mutants selected from M_2 population during 2002-2003 (crosses made in 2000-2001)

Parentage	Mutant selected (No.)	Plant height (cm)	CLCuV reaction	Boll weight (g)	Yield/ plant (g)
DNH-49XPIM-98	20	105-111	R	4.5	285
CIM-1100XPIM-77-2	30	123-180	R	4.8	290
CIM-435XDNH-49	10	118-165	R	4.9	300
VH-137XPIM-77-2	13	100-208	R	4.7	275
BH-36XBH-36	3	125-183	R	4.9	305
CIM-473	-	165	R	4.3	150
Susceptible line	-	105	HS	4.0	105

Table VII. Maximum yield of different crosses of 2001-2002 with irradiated pollen in M_1 generation during 2002- 2003

Crosses	CLCuV disease reaction	Yield/ plant (g)	Remarks
VH-137 x K-599	R	290.0	Early short inter node
K-599 x CIM-1100	R	261.0	Medium symp. Early
DNH-49 x CIM-443	R	300.0	Better boll opening
CIM-443 x L-31	R	200.0	Good boll size early
K-599 x VH137	R	250.0	Good opening
VH-137 x K-599	R	250.0	Medium maturity
CIM-435 x K-599	R	192.0	Tall medium late
DNH-49 x K-599	R	235.0	Tall late
CIM 473	R	148.0	-
Susceptible line	HS	105.0	-

Table VIII. Characteristics of promising advanced mutants developed before the project with irradiated pollen during 2002-2003.

Mut. /Var.	CLCuV disease reaction	Boll weight (g)	Yield ha^{-1}	% increase in yield over CIM-473
A. First Experiment				
NIAB-98	R	3.5	5583.9	36.02
NIAB-829	R	4.5	5408.9	31.76
NIAB-824	R	4.5	4598.0	12.00
CIM-473	R	4.0	4105.0	-
B. Second Experiment				
NIAB-869	R	3.5	4625.5	28.28
NIAB-785	R	4.5	6406.1	34.30
NIAB-846	R	4.5	5996.1	64.66
CIM-473	R	4.0	4105.0	-

R= resistant to CLCuV; HS= highly susceptible to CLCuV

studied in comparison to the newly approved standard cotton variety CIM-473 in two different experiments. In the first experiment all of the mutants (NIAB-98, NIAB-829, NIAB-824) gave higher yield than CIM-473. The maximum yield was given by mutant NIAB-829 (6406.1 kg/ha). The percent increase in yield over standard (CIM-473) ranged from 12.0 to 36.02. Similarly in the second experiment all of the mutants (NIAB-869, NIAB-785, NIAB-846) gave higher yield than CIM-473. The maximum yield was given by mutant NIAB-846 (4380.9 kg/ha). The percent increase in yield over standard cotton variety (CIM-473) ranged from 28.28 to 64.66.

The results reported above have clearly indicated that from a very small M_2 population i.e. about 700 plants, higher rate of mutations was achieved through pollen irradiation, therefore our results have clearly confirmed the earlier findings (Miah & Yamaguchi, 1965; Vig, 1973; Wang, 1990). Furthermore, the method of gamete treatment was easier to apply than that of zygote/seed treatment. Similarly, from the irradiation pollen before cross-pollinations resulted in the induction of mutations in cotton (Pate & Duncan, 1963; Krishnaswami & Kothandaraman, 1976). Moreover these results have clearly confirmed and supported that the treatment of pollen with of low doses of gamma rays before cross-pollinations are suitable to induce useful genetic variability in cotton (Aslam & Stelly, 1994; Aslam *et al.*, 1994; Aslam, 2000, 2002).

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