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Induction and Early Evaluation of a High Yielding Elite Cotton Mutant Line, PIM-76-8 Through the Use Pollen Irradiation Technique

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Abstract: M_2 population was raised from M_1 seed developed from the intraspecific crosses (NIAB-78 x REBA–288) with male parent pollen irradiated at 10Gy of gamma rays. Different 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 segregating generations in comparison to then standard cotton varieties. Out of these, the mutant PIM-76-8 consistently out-yielded all the prevalent standard cotton varieties/parents i.e. CIM-240, CIM-448, CIM-443, NIAB-78 and Reba-288. On the average it gave 28.2% higher yield than the standard cotton variety CIM-443 in the micro-yield trials conducted at NIAB during 1998-99. Further evaluation of mutant, PIM-76-8 is in progress and the results are encouraging.

Key words: Cotton, PIM, induction, pollen irradiation technique, early evaluation, mutant

Introduction

Although the conventional breeding approaches are the most common methods to be employed for the improvement of crop plants. But the success of all these approaches is very likely to be depended upon the availability of genetic variability present within the existing germplasm at hand. However if the desired trait is not present or linked with other unwanted traits in the existing germplasm, then the crossbreeding may not be worthwhile. In such cases recombination of genes is to be sought out to achieve the desired objectives. Recombination is a key process in the creation of genetic variation and the recombination of linked genes is brought about by crossing over. In general 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, ultimately is expected to further increase the variability in F_2/M_2 population. Increased variability in F_2M_2 for quantitative traits has been reported in rice (Jalil Miah and Yamaguchi, 1965). Similarly radiation as well as several chemicals are reported to increase somatic recombinations (Vig, 1973). Exposure of seed to ionizing radiations 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 (Carneiius, 1973; Micke and Donini, 1982; Micke *et al*., 1987; Iqbal *et al.,* 1991, 1994).

Treatment of gametes with irradiation resulted in a lower chromosome aberration rate in the M_1 generation and a higher mutation frequency and wider spectrum in the M_2 generation (Shi *et al*., 1987). The favourable mutation frequency was higher than that of seed treatment (Xu *et al.,* 1985). The method of gametes treatment was 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). The studies carried out by Aslam and Stelly (1994) and Aslam *et al.* (1994) have shown that treatment of pollen with low doses of gamma rays (5Gy to 20Gy) before cross-pollinations are suitable to induce useful genetic variability in cotton. The objectives of the present research studies were to induce genetic variability through crosses with irradiated male parent pollen and consequently to select and to evaluate the desirable recombinants in the subsequent segregating generations.

Materials and Methods

A higher yielding and locally well-adapted cotton variety NIAB-78 and an exotic line Reba -288 belonging to *G. hirsutum* were used as parents in these studies. NIAB-78 was used as male parent, while Reba-288 was used as female parent. Male parent pollen was irradiated at 5 Gy and 10 Gy of gamma rays before cross-pollinations and crosses were made, using a simple artificial cross-pollination technique developed by Doak (1934), to develop M_0 seed. 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 cm 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 of one locule from each boll of each M_1 plant separately for studying it as M_2 generation. The M_2 population comprising of about more than three hundred individual plants were studied and selections for the desirable mutants/recombinants were carried out. Both the M_1 and M_2 populations were exposed to CLCuV disease under natural infestation during 1994 and 1995, respectively, using spreader rows of highly susceptible cultivar S-12 to encourage uniform inoculation. Highly susceptible cultivar S-12 received 100 percent disease infestation and the disease intensity was measured as described by Siddig (1968). The selection from the M_2 population was based upon, early maturity, better plant type, higher yield/better yield components etc. alongwith resistance to CLCuV disease. Of these, 12 promising mutants were grown in M_3 generation as plant progeny rows to study their breeding behaviour. The size of the individual plot was 0.75m x 10m. The breeding behaviour of these progenies was studied and consequently from the M_3 generation, one higher yielding progeny, PIM-76-8 was finally selected and studied in M_4 generation to see its breeding behavior/uniformity and also to confirm its higher yield potential. Finally an almost uniform progeny was bulked and evaluated for yield potential in macro-yield trials in comparison to prevalent standard cotton variety CIM-443 at NIAB, Faisalabad during the year 1998-99. The standard agronomic practices and plant protection measures were adopted every year throughout the crop-growing season.

Results and Discussion

The results indicated that M_1 generation plants were more vigorous in growth and depicted hybrid vigour for various traits and also showed resistance against CLCuV disease under severe natural disease epidemic conditions, i.e., where the highly susceptible cotton variety S-12 had 100 per cent CLCuV disease infestation. The M_2 generation results revealed that the 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 intensity. The M_2 mutants had better boll weight and higher yield as compared to both the parents. The boll weight and yield of the promising mutants selected from M_2 population ranged from 3.5 to 5.0 grams and 211 to 396 grams respectively (Table 1). Whereas the boll weight and yield of both the parents i.e., NIAB-78 and Reba 288 ranged from 3.0 to 3.5 grams and 93 to 160 grams per plant respectively. Promising mutants selected from M_2 generation were studied in plant progeny rows to see their breeding behavior and yield potential. The results obtained from M_3 generation indicated that some of the progenies showed segregation for CLCuV disease resistance, but most of the progenies preserved their better boll weight and higher yield potential over both the parents. The yield of these progenies ranged from 1039.1 to 2185.5 Kg ha^{-1}. Whereas the standard cotton variety CIM-240, then gave yield of 774.5 Kg ha^{-1}. On the basis of higher yield along with other desirable traits, finally six mutant progenies were selected for further evaluation in comparison to standard cotton variety CIM-448 in M_4 generation. The M_4 generation results revealed that the mutant progeny PIM-76-8 gave the yield of 2081 Kg ha^{-1} and ranked second as compared to all the other mutant progenies and gave 32.3 percent higher yield than the then standard cotton variety CIM-448 and also showed resistance to CLCuV disease. Moreover this progeny, i.e., PIM-76-8 was found almost uniform and therefore was bulked and evaluated in micro-yield trials as M_5 generation to confirm its yield potential at NIAB. The results indicated that on the average the mutant PIM-76-8 gave 28.2 percent higher yield than the prevalent standard cotton variety CIM-443, resistant to CLCuV disease and gave much higher yield i.e., 100 percent than standard, NIAB Karishma tolerant to CLCuV disease (Table 2-4). The average yield of the mutant PIM-76-8 was 3304.9 Kg ha^{-1}, whereas CIM-443 had yield of 2578.8 Kg ha^{-1} and NIAB Karishma, produced 1467 Kg ha^{-1} yield of seed cotton.

As in case of seed irradiation one has to irradiate the whole genome thereby disturbing all the genetic makeup of the treated individual. As a result most of the changes are the somatic ones and hence are non-heritable. Therefore large M_2 population is required i.e., some time may be more than 12,000 individual plants to obtain desirable genetic mutations (Iqbal *et al.,* 1994). It is obvious that pollen irradiation is a valuable technique, which can be employed to improve crop plants. Since irradiated pollen is a germ cell and after fertilization the zygote has only half of the genome, which receive the irradiation, hence the major changes are minimal, as observed in case of seed irradiation. Incase of seed irradiation usually from each M_1 plant seed cotton from one locule per boll is collected and then pooled to have M_1 seed to grow M_2 population. But here we grew each M_1 plant separately in plant progeny rows as M_2 population to carry out selection. With this there was clear-cut depiction of the expected obvious changes in each M_2 progeny and the progenies were of varied nature as expected in M_2 generation due to segregation. 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 helped to achieve uniformity earlier as compared to seed irradiation. Moreover as the male gamete is irradiated with low doses before fertilization hence therefore more recombinations are brought about due to enhanced chaisemata formation/crossing over during meiotic stages of cell divisions. The results reported above has shown that from a very small M_2 population i.e., about 1000 plants, higher rate of mutations was achieved through pollen irradiation, therefore our results have clearly confirmed the earlier findings (Jalil Miah and Yamaguchi, 1965; Vig, 1973; Shi *et al*., 1987; Xu *et al*., 1985). The method of gamete treatment was 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). The studies carried out by Aslam and Stelly (1994) and Aslam *et al.* (1994) have shown that treatment of pollen with of low doses of gamma rays (5 Gy to 20 Gy) before cross-pollinations are suitable to induce useful genetic variability in cotton. On the basis of higher yield potential and desirable other economic characters, the further evaluation of the mutant PIM-76-8, now named as NAIB-98, in the zonal yield trials is in progress. Moreover true to type/ breeders seed production of the mutant is being followed up through developing SPPR is also in progress.

Table 1: Characteristics (range) of the promising mutants selected from M_2 Population from crosses with irradiated pollen during 1995-96

Parentage	Promising mutants selected (No)	Plant height (cm)	CLCuV reaction (Grade)*	Boll weight (gram)	Yield/plant (gram)
NIAB-78 x Reba 288 (M_2 generation)	12	102-184	(0-1)	3.5-5.0	211-396
NIAB-78	Female parent	135	(4)	3.0	150
Reba-288	Pollen parent	160	(0-1)	3.5	93

*Rating Scale 0-5, 0 = Immune 0-1 = Resistant 5 = Highly susceptible

Table 2: Characteristics of promising M_3 mutant progenies from crosses with Irradiated pollen during 1996-97

Mut./Var.	Plant height (cm)	CLCuV reaction (grade)*	Boll weight (gram)	Yield ha^{-1} (Kg)
PIM-76- 1	184	(0-2)	4.7	1848.7
" 2	168	(0-2)	4.3	2185.5
" 3	102	(0-2)	3.9	1539.5
" 4	86*	(0-2)	3.5	1334.6
" 5	138	(0-2)	4.7	1260.8
" 6	116	(0-2)	5.0	2017.4
" 7	120	(0-1)	4.8	1625.1
" 8	120	(0-1)	4.0	2017.4
" 9	152	(0-1)	4.2	1039.1
" 10	122	(0-1)	4.8	1628.0
" 11	128	(0-2)	4.7	2017.4
" 12	112	(0-2)	4.7	2129.4
CIM-240 standard	125	(2-3)	4.0	774.5
S-12 *	135	(5)	4.0	514.5

Rating Scale 0-5, 0 = No symptoms, 0-1 = Resistant 5 = Highly susceptible * = Highly susceptible

Table 3: Characteristics of the promising plant progenies of mutant PIM-76 in M_4 generation during 1997-98

Mutant/Var.	Plant height (cm)	CLCuV infestation (grade)*	Boll weight (gm)	Yield ha^{-1} (kg)	Fibre		
					length (mm)	fineness (µg/in)	maturity (%)
PIM-76-2	114	(0-2)	4.2	2228	28.6	4.6	92
PIM-76-6	130	(0-2)	4.3	1492	27.5	4.8	90
PIM-76-8	125	(0-1)	4.7	2081*	27.0	4.7	94
PIM-76-1	139	(0-2)	4.1	1895	29.0	4.3	85
PIM-76-11	132	(0-2)	4.0	1329	26.9	4.7	92
PIM-76-12	134	(0-2)	4.4	1823	26.7	4.7	92
S-12**	160	(0-2)	4.0	1067	28.0	5.0	90
CIM-448***	145	(0-2)	3.3	1573	27.0	4.9	91

*Yield increase of PIM-76-8 over latest standard variety CIM-448 is 32.3%

*Rating Scale =0-5 0=Immune 0-1=Resistant 5=Highly susceptible to CLCuV.
** S-12 = Highly susceptible to CLCuV *** CIM-448 = Latest standard variety, resistant to CLCuV

Table 4: Performance of mutant PIM-76-8 (average of three experiments)in Micro-yield trials incomparison to CIM-443 at NIAB during 1998-99

Mut./Var.	CLCuV disease reaction (grade)*	Boll weight (gram)	Yield ha^{-1} (Kg)	Percent Increase over
PIM-76-8	(0-1)	4.0	3304.9	-
CIM-443	(0-1)	3.1	2578.8	+28.2
NIAB-Karishma*	(3-4)	3.2	1467.0	
S-12**	(5)	4.0	995.0	

Rating Scale 0-5, 0 =Immune, 0-1= Resistant, 5= Highly susceptible
*Standard variety tolerant to CLCuV disease ** Highly susceptible

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