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# Utilization of Pollen Irradiation Technique for the Improvement of *G. hirsutum* L.

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**Abstract:** The pollen irradiation technique was employed for the induction of genetic variability leading to improve the genetic potential of cotton, *G. hirsutum* L. Consequently different mutants were selected from $M_2$ population; derived from crosses (D 104-1-5 x Reba 288, CIM-240 x Reba 279, NIAB-78 x Reba 288 etc.) with irradiated (5-10Gy) pollen. The selected mutants possessed higher yield potential, had big boll size, early in maturity, had shorter internodal length etc. along with resistant to CLCuV disease. These $M_2$ mutants when further evaluated for higher yield potential etc. showed segregation for different economic traits in $M_3$ generations. Out of these, the mutants PIM-77, PIM-78, PIM-80 etc. consistently out-yielded all the prevalent standard cotton varieties/parents i.e. CIM-240, CIM-443, and NIAB Karishma etc. The maximum average yield of 3745kg/ha was given by mutant PIM-80, with an overall increase of 52 per cent over the latest standard cotton variety CIM-443 having yield of 2475 kg/ha. Further evaluation of mutants to achieve uniformity /stability for various economic traits is being carried out.

**Key words:** Utilization, pollen irradiation technique, cotton, improvement, *G. hirsutum*

## Introduction

The 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).

The treatment of gametes before fertilization with irradiation had a lower chromosome aberration rate in the $M_1$ generation, a higher mutation frequency and wider spectrum in the $M_2$ generation as compared to seed irradiation. The favourable mutation frequency was also higher than that of seed treatment (Wang, 1990). Moreover the method of gametes treatment was easier to apply than that of zygote/seed treatment. The 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. The increased variability in $F_2M_2$ for quantitative traits has been reported in rice (Jalil Miah and Yamaguchi, 1965).

The irradiation of male parent pollen before cross-pollinations resulted in the induction of mutations in cotton (Pate and Duncan, 1963 and Krishnaswami and Kothandaraman, 1976). The studies carried out by Aslam and Stelly (1994), Aslam *et al.*, (1994) and Aslam and Elahi, (2000) have shown that treatment of pollen with low doses of gamma rays (5Gy to 10Gy) before cross-pollinations are suitable to induce useful genetic variability in cotton. Similarly besides radiation the several chemicals are reported to increase somatic recombinations (Vig, 1973).

The objectives of the present research studies were to induce genetic variability through crosses with irradiated male parent pollen and consequently to select the improved genotypes of cotton from the subsequent segregating generations.

## Materials and Methods

Two local cotton varieties i.e. NIAB-78, CIM-240 and a transformed line D104-1-5 (Aslam *et al.*, 1997) were used as female parents, while exotic genotypes, Reba B.J-50, S.-J-5 etc. belonging to *G. hirsutum* were used as pollen parents. Before cross-pollinations the male parents pollen was treated with of gamma rays (5 Gy and 10 Gy) and following crosses were attempted during the year 1994-95, using a simple artificial cross-pollination technique developed by Doak (1934).

i) D-104-1-5 x Reba 279
ii) NIAB-78 x Reba 288
iii) CIM-240 x Reba 288
iv) NIAB-78 x Reba B.J-50
v) S.J.-5 x Reba 279
vi) NIAB-78 x Reba B. J-50

At maturity the seed cotton was collected from the treated bolls of all the above mentioned crosses and was ginned to develop $M_0$ seed. The $M_1$ 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. The $M_2$ population was grown from $M_1$ generation seed. The $M_2$ population comprising of about more than one thousand individual plants were studied for carrying out selection for the desirable mutants/recombinants. Both the $M_1$ and $M_2$ populations were exposed to CLCuV disease under natural infestation during the year, 1995 and 1996, respectively, using spreader rows of highly susceptible cultivar S-12 to encourage uniform inoculation. Highly susceptible cultivar S-12 received 100 per cent disease infestation and the disease intensity was measured as described by Siddig (1968). From $M_2$ population various promising mutants were selected on the basis of field performance. The selections from the $M_2$ population was based upon, big boll size/good opening, better plant type, alongwith higher yield and resistance to CLCuV disease etc. Out of these about 50 finally selected promising mutants were grown as plant progeny rows in $M_3$ generation, during the year 1997-98, to study their breeding behaviour. The size of the individual plot was 0.75m x 10m. The different promising $M_3$ generation mutants i.e.PIM-80-, PIM-77, PIM-78 etc-were studied in M4 generation to see their breeding behavior/uniformity and also to confirm their other better characteristics in comparison to NIAB Karishma and CIM-443 during the year 1998-99. The standard agronomic practices and plant protection measures were adopted throughout the crop-growing season during the respective years.

## Results and Discussion

The $M_1$ population manifested hybrid vigour for various plant characteristics alongwith 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 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. Different mutants having high b oll bearing , early maturity and resistance to CLCuV disease alongwith other desirable traits were selected. These $M_2$ mutants possessed better boll weigh and had higher yield as compared to standard cotto varieties and the respective parents. The boll weight an yield per plant of the promising $M_2$ mutants ranged from 3. to 5.1 grams and 169 to 352 grams respectively (Table 1) Whereas the boll weight and yield per plant of the parent and standard cotton varieties ranged from 3.0 to 4.5 gram and 50 to 250 grams respectively. Promising mutant selected from $M_2$ generation were studied as plant progen rows to see their breeding behavior and to confirm highe yield potential in $M_3$ generation during 1997-98. The result indicated that all the mutants gave higher yield than th standard cotton varieties (NIAB-78 and CIM-448). Thes mutant were also found resistant to CLCuV disease. Th mutant PIM-80 had the maximum (average) yield of 400 kg/ha with an overall increase of 56 per cent over th higher yielding standard cotton variety, CIM-448 (Table 2) While the mutant PIM-78 ranked second and gave th average yield of 3772 kg/ha. The yield of the standar cotton varieties i.e NIAB-78 and CIM-448 ranged from 1997.5 to 2575.0 kg/ha respectively. Moreover some o

Table 1: Characteristics of promising $M_2$ mutants selected from crosses with Irradiated pollen during 1996-97

| Mutants Selected Name | (No) | Parentage | Radiation dose (Gy) | CLCuV reaction | Boll weight (grams) | Yield/plant (grams) |
|---|---|---|---|---|---|---|
| PIM-75 | 3 | D-104-1-5* x Reba 279+ | 5.0 | R | 3.8-5.1 | 177-350 |
| PIM-76 | 5 | NIAB-78* x Reba 288+ | 10.0 | R | 3.2-5.0 | 185-250 |
| PIM-77 | 12 | NIAB-78* x Reba 288+ | 5.0 | R | 3.5-5.1 | 186-345 |
| PIM-78 | 12 | NIAB-78* x Reba B.J-50+ | 10.0 | R | 3.8-4.8 | 169-352 |
| PIM-80 | 7 | S.J.-5* x Reba 279+ | 5.0 | R | 3.7-5.3 | 186-290 |
| PIM-79 | 6 | NIAB-78* x Reba J-50+ | 5.0 | R | 4.2-4.5 | 175-242 |
| NIAB-78 | | parent | | S | 3.0 | 120 |
| CIM-240 | | standard | | T | 4.0 | 165 |
| Reba B J-50 | | non adaptive | | R | 4.0 | 50 |
| Reba-288 | | non adaptive | | R | 3.5 | 95 |
| Reba 279 | | non adaptive | | R | 4.0 | 80 |
| S.J.-5 | | non adaptive | | R | 4.0 | 80 |
| D-104-1-5** | | transformed line | | T | 4.5 | 250 |
| S-12 | | | | HS | 4.0 | 90 |

* Female parent + Pollen parent R=Resistant to CLCuV S = susceptible to CLCuV HS= Highly susceptible to CLCuV T= Tolerant
** A transformed line obtained by injecting *G.barbadense* DNA *into G.hirsutum*

Table 2: Characteristics of the promising plant progenies of big boll mutants selected from $M_3$ generation during 1997-98

| Mutants/.Progenies | Elite/desirable progenies (No) | Plant height (cm) | CLCuV infestation | Boll weight (gm) | Yieldha$^{-1}$ (kg) | % increase over CIM-448 |
|---|---|---|---|---|---|---|
| PIM-80 | 3 | 151 | R | 4.2-4.5 | 4004.0 | 56.0 |
| PIM-79 | 1 | 106 | R | 5.0 | 3544.9 | 34.0 |
| PIM-78- | 4 | 142 | R | 4.7-4.4 | 3772.0 | 47.0 |
| PIM-75- | 3 | 141 | R | 4.4 | 3358.0 | 31.0 |
| PIM-86 | 2 | 145 | R | 4.4 | 3595.0 | 40.0 |
| PIM-77 | 2 | 150 | R | 4.5 | 3271.0 | 27.0 |
| NIAB-78 | | 130 | S | 3.0 | 1997.5 | - |
| CIM-448** | | 150 | R | 3.2 | 2575.0 | - |
| S-12 | | 135 | HS | 3.5 | 1103.0 | - |

R=Resistant to CLCuV S = susceptible to CLCuV HS=Highly susceptible to CLCuV
** CIM-448 = Latest standard variety, resistant to CLCuV

Table 3: Characteristics (average) of the promising big boll mutants/progenies selected from $M_4$ generation during 1998-99

| Mut./ prog. | Elite/Desirable Progenies (No) | Plant height (cm) | CLCuV disease reaction | Boll weight (gram) | Yieldha$^{-1}$ (Kg) | % Increase over CIM-443 |
|---|---|---|---|---|---|---|
| PIM-80 | 3 | 153 | (R) | 4.5 | 3745 | 52 |
| PIM-77 | 3 | 146 | (R) | 4.4 | 3588 | 45 |
| PIM-78 | 4 | 148 | (R) | 4.4 | 3259 | 32 |
| PIM-86 | 2 | 148 | (R) | 4.5 | 3500 | 42 |
| CIM-443 | | 119 | (R) | 3.0 | 2475 | - |
| NIAB-Karishma* | | 158 | (T) | 3.3 | 2390 | - |
| S-12** | | 146 | (HS) | 4.5 | 1598 | - |

R= Resistant to CLCuV, HS= Highly susceptible to CLCuV * = Standard variety tolerant to CLCuV ** Highly susceptible to CLCuV the the $M_3$ generation progenies revealed segregation for CLCuV disease resistance, but most of the progenies preserved their better boll weight and higher yield potential as compared with the standard cotton varieties. On the basis of higher yield alongwith other desirable traits, finally selected mutant progenies were evaluated in comparison to standard cotton varieties (NIAB-Karishma and CIM-443) in $M_4$ generation. These higher yielding mutant progenies maintained their higher yield potential over the latest standard cotton varieties by giving average yield of 3259 to 3745 kg/ha. Whereas the yield of the standard cotton varieties i.e. NIAB-Karishma and CIM-443 ranged from 2390 to 2475 kg/ha respectively (Table 3). The maximum average yield of 3745kg/ha was given by mutant PIM-80, with an overall increase of 52 per cent over the latest standard cotton variety CIM-443. However most of the $M_4$ generation progenies were not uniform for all the characters and hence the further studies are being continued to achieve uniformity for different economic traits.

As the whole genome is to be irradiated, in order to create genetic variability through seed irradiation, which ultimately disturb the whole genetic makeup of the treated individual. As a result most of the changes occurring are the somatic changes 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 quite obvious that the pollen irradiation is a valuable technique, which can be employed to improve crop plants. 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. 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. But by following the pollen irradiation technique each $M_1$ plant has to be grown separately as plant progeny rows to develop $M_2$ population to carryout selection. With this there is clear-cut depiction of the expected obvious changes in each $M_2$ progeny and the progenies are always of varied nature as expected in $M_2$ generation due to segregation/mutations. 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 early as compared to seed irradiation. Since the male gamete is irradiated with low doses before fertilization and therefore more recombinations are brought about due to enhanced chaisemata formation/crossing over during meiotic stages of cell divisions. The results reported above have clearly indicated that from a very small $M_2$ population i.e. about 1000 plants, higher rate of mutations was achieved through pollen irradiation, therefore these results have clearly confirmed the earlier findings ((Jalil Miah and Yamaguchi, 1965, Vig, 1973, Wang, 1990). Furthermore 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 and Krishnaswami and Kothandaraman, 1976). These results have clearly confirmed and supported that the treatment of pollen with low doses of gamma rays (5 Gy to 10 Gy) before cross-pollinations are suitable to induce useful genetic variability in cotton (Aslam and Stelly (1994), Aslam *et al.* (1994) and Aslam and Elahi (2000).

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