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Full Length Article

NIAB-2008: A New High Yielding and Long Staple Cotton Mutant Developed through Pollen Irradiation Technique

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Abstract

This paper describes about development of cotton mutant NIAB-2008, developed through pollen irradiation technique with improved yield and fibre quality. A local variety NIAB-78 was crossed with exotic line REBA-288 using irradiated pollen @10 Gray (Gy) of gamma rays before cross pollination. The purpose of the study was to create new genetic variability and select the desirable new cotton mutants. After irradiation and hybridization different generations were raised to evaluate the effect of irradiation treatment and induction of mutations. Significant variations from control/parents were observed in evaluated generations. From M_1 seed, the M_2 population was raised and different desirable mutants were selected. The selected mutants were evaluated in mutated generations (M_3-M_6) for different yield contributing traits and yield potential along with fibre quality and variations from parents were observed. From selected mutants, an elite mutant i.e., M-7/09, later named as NIAB-2008 was finally selected. It was evaluated for seed cotton yield, adaptability, resistance/tolerance to diseases, and fibre quality in different trials. It produced 32.9% higher seed cotton yield compared to standard CIM-496 at local NIAB trials. It produced 32.7% and 32.2% higher seed cotton yield compared to standard in provincial coordinated cotton trials (PCCT) and in national coordinated varietal trials (NCVT) respectively. The mutant NIAB-2008 has desirable fibre quality traits i.e., ginning out turn (GOT) 37.99%, fibre length 31.16 mm (long staple category), fibre fineness 4.74 µg/inch, uniformity index 83.5%, fibre maturity 80.5% and fibre strength 92.2 thousand pounds per square inch (TPPSI). Its distinguish characteristics are early maturity, high yielding, short stature, good boll bearing and better tolerance to cotton leaf curl virus-Burewala strain (CLCuV-B) disease. From these results it is concluded that low dose pollen irradiation technique has effectively stimulate/increase the different yield and yield contributing traits, fibre quality and tolerance to diseases in cotton and this technique is proved economical.

Keywords: Pollen irradiation; Yield; Fibre quality; Long staple; Cotton; NIAB-2008

Introduction

Cotton is an important cash crop of Pakistan, contributes a major share in foreign exchange earnings and provides the basis for a national textile industry. Cotton (*Gossypium hirsutum* L.) is cultivated on an area of 3125, 000 ha with an annual production of 12.8 million bales (Anonymous, 2013-2014). Overall, the living of millions of people in Pakistan is linked with cotton cultivation, ginning, oil industries, trade and spinning processes. It has many uses but it is mainly cultivated for its fibre and seed oil (Pandey, 1998). *Gossypium hirsutum* (upland cotton) is mainly cultivated in the world and produced 90% of world cotton production followed by 8% from *G. barbadense* (american pima or egyptian cotton) and 2% from (asiatic, desi cotton) *G. herbaceum and G. arboreum* (Wendel and Cronn, 2003).

Lot of efforts has been made by cotton researchers to develop upland cotton varieties having high yield potential, desirable fibre quality and tolerance/resistance to insect's pests and diseases through conventional breeding approaches. Success in this regard is achieved but the textile mills have been increased from 2 to over 500 in Pakistan. It is estimated that our textile industry would require 20 million bales of lint by 2020. However, cotton producers in Pakistan are currently faced with rising production costs and static return (Haidar *et al*., 2007).

Moreover, there are limitations of availability of sufficient genetic variability in the native germplasm (Haidar *et al*., 2012). To achieve desired objectives through conventional breeding approaches is highly depended on adopted techniques. Raising of induced variant/mutants populations with proper screening techniques is useful to identify the desirable traits among the mutant populations and to evaluate their adaptability (Sikora *et al*., 2011).

Different types of physical and chemical mutagens are extensively used to induce variations in plants. Among the physical agents, gamma rays are used to create point mutations and small deletions (Wu *et al.,* 2005).

The mutation breeding techniques have been effectively used for the improvement of yield characters and development of germplasm with novel and desired traits of major crop plants (Maluszynski *et al.*, 1995; Shu and Lagoda, 2007; Sestili *et al.*, 2010; Tomlekova, 2010). Number of mutants of different crop plants have been developed and released in different countries of the world with improvement in some characters (Ahloowalia *et al.*, 2004). Such mutants are reported in cotton (Muthusamy and Jayabalan, 2007; Muthusamy and Jayabalan, 2011), soybean (Hofmann *et al.*, 2004), potato (Li *et al.*, 2005), cassava (Joseph *et al.*, 2004, Buttibwa *et al.*, 2015), *Chrysanthemum* (Datta *et al.*, 2005) and groundnut (Muthusamy *et al.*, 2007).

The approach like, the exposure of seed to ionizing radiations in cotton is used by different researchers. A new cotton variety MCU-7 was developed, which was early, high yielding and has long staple then its parents (Carnelius, 1973). Miah and Yamaguchi (1965), reported increase in genetic variability for different quantitative traits in segregating F_2/M_2 population in rice. Mike *et al*. (1987) also reported seed irradiation studies in various crops i.e., wheat, barley, rice, maize etc in different countries**.** A cotton mutant NIAB-92 was developed through seed irradiation technique which was early and high yielding as compared to parent Stoneville-231 (Iqbal *et al*., 1994). Through F_1 seed irradiation a high yielding and early maturing mutant NIAB-78 was also developed (Iqbal *et al*., 1991). Another technique i.e. treatment of pollen with gamma rays is also utilized by different researchers.

Sanamian (2003) reported various genomic and chromosomal mutations in M_2 families developed through pollen irradiation. Variability in cotton plants treated with pollen irradiation is mainly because of chromosomal rearrangements and genomic mutations during meiosis. Vig (1973) reported that radiation treatments enhance crossing over near the centromere region in *Glycine max*. Moreover radiations and chemicals treatments increase somatic recombinations. Pollen irradiation before cross-pollinations for hybridization in different crops has also reported by different researchers (Pate and Duncan, 1963; Ibragimov *et al.*, 1965; Krishnaswami and Kothandaraman, 1976; Aslam *et al.*, 1994; Aslam and Stelly, 1994; Aslam, 2002) and is considered useful to create new genetic variability in cotton.

This research study was conducted to create genetic variability by gamma irradiation, selection of mutants with desirable economic traits and confirmation of stability, adaptability of the selected mutant with better yield and quality characters. This manuscript details the report on use of low dose of induced mutations on germ cells and selection of useful mutants.

Materials and Methods

Plant Material

The cross was made by utilizing NIAB-78 (local cotton variety) as female parent with REBA-288 (exotic line) at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. The local variety NIAB-78 has good agronomic traits along with better yield potential, average quality parameters but susceptible to cotton leaf curl virus disease. Whereas the exotic line REBA-288 has bushy type plant, average quality parameters but better tolerance/resistance to cotton leaf curl virus disease. Selfed seeds of male and female parents were grown and approximately fifty plants of each were developed. At maturity flower buds of female parent were emasculated and covered with paper bags at evening time. Flower buds of male parent were also covered to protect any mixing.

Radiation Treatment

Male parent pollen was collected from covered flowers after anthesis and irradiated with gamma rays from a ^{60}Co irradiation source. The irradiation was performed at room temperature at NIAB, Faisalabad, Pakistan during 1998-1999. Emasculated flowers were pollinated with irradiated pollen and rebagged to prevent uncontrolled crossing. Bolls developed from the crossed flowers were harvested and seeds were obtained and designated as M_0 seed.

Evaluation of Mutated Generations

M_1 population was developed from M_0 along with both parents as control at experimental field of NIAB, Faisalabad. The seeds were planted at a spacing of 30 x 75 cm plant to plant and row to row respectively. The M_2 population was raised from M_1 generation and it consists of more than one thousand individual plants. In M_2 generation, maximum numbers of mutants/recombinants were selected keeping in view of different plant traits. Selected mutants were planted in M_3 generation in replicated progenies. These progenies were studied in M_3 generation for their breeding behavior and economic traits and one elite line was selected. Plant progeny rows were also studies in M_4 generation to confirm desirable traits. The progeny M-7/09 was selected from M_6 and bulked for evaluation as NIAB-2008. All these generations were raised and evaluated at NIAB during 2000–2006, where the soil type is of clay loam. Agronomic and plant protection practices were carried out throughout the crop growing season during respective years.

Evaluation in Adaptability Trials

Various yield trials (local, zonal i.e., NCVT, PCCT, 1.25 acre PSC farm etc.) were conducted at public sector experimental institutes etc during 2007–2012. The objective was to analyze yield potential, fibre quality traits and wider adaptability at different environments and soil types. It was also evaluated for their response to earliness, insects and diseases particularly of CLCuV-B.

Screening for Cotton Leaf Curl Virus Disease and Insect Pests

Screening against cotton leaf curl disease was done through grafting and whitefly inoculation technique. For grafting studies, ten pots for each variety were sown in glass house. Plants were graft inoculated with CLCuV-B following the methodology of bottle shoot grafting method (Akhtar *et al*., 2002, 2010). Entomological studies regarding its response to sucking pests and damage by bollworms were conducted under optimized spray conditions at NIAB Faisalabad.

Fibre Characters Analysis

Fibre characters of the selected mutant lines were analyzed using High Volume Instrument (HVI) as well as manually operated instruments at NIAB, Faisalabad. As per requirement of mandatory evaluation by Punjab Seed Council (PSC), fibre quality of NIAB-2008 was also got analyzed from four standard laboratories i.e. Cotton Research Institute (CRI), Faisalabad; NIBGE, Faisalabad; All Pakistan Textile Mills Association (APTMA), Lahore and CCRI, Multan. The samples were collected by members of expert sub committee (ESC) of PSC during spot examination and fibre characters in the standard labs were also analyzed using High Volume Instrument (HVI). These fibre characters were evaluated by expert subcommittee members during 2014-2015.

Statistical Analysis

The experiments related to yield evaluation were also planted in randomized completed block design (RCBD) with three replication and different number of treatments/varieties during different years. The data for different morphological characters and seed cotton yield in different yield trials and fibre characters were subjected to analysis of variance (ANOVA) using the methodology (Steel *et al.*, 1997). In addition data for seed cotton yield in various adaptability trials were compared using Fisher's least significant difference (LSD) procedure.

Results

A local cotton variety was crossed with an exotic line by using gamma irradiated pollen and a useful mutant NIAB-2008 was selected. Its developmental history is given (Table 1). The mutant NIAB-2008 (Fig. 1A) showed early flowering, medium height, higher number of bolls per plant, more number of nodes/sympodial branches, good /fluffy opening, better yield potential and plant type as compared to parents NIAB-78 (Fig. 1B) and REBA-288 (Fig. 1C). Data on different traits of selected mutant along with control lines is given in detail and discussed.

Influence of Mutagenic Treatments on Selected Plants

Significant differences were observed between the control and plants developed from the irradiated pollen. In M_1 generation the plants were taller in growth and showed hybrid vigour for different traits as compared to the non-irradiated both parents/control. In M_1 generation, the germinated plants showed better tolerance to cotton leaf curl virus disease (CLCuV-B). There was variation in M_2 generation and plants possessing good characters were selected. Succeeding generation M_3-M_6 was evaluated, and as a result mutant with high yield potential was selected, which was later named as NIAB-2008. The comparison of selected mutant from parents (control) showed differences in plant traits (Table 2). The plants developed from the treated pollen showed different developmental plant features at seedling and normal plants development stages in the field conditions. Similar results were earlier observed in cotton (Muthusamy and Jayablan, 2000, 2001).

Field Evaluation of Mutant Line

A range of morphological variations was observed from seedling stage to maturity in M_2, M_3 and M_4 generations. Plants with improved yield and yield contributing traits and ginning out turn percentage, staple length, fibre fineness, fibre strength and uniformity ratio etc were selected to record and analyze the effect of gamma rays treatments on the selected plants from the control (parents etc). The flowering periods of mutants were decreased compared to the parents whereas increase in seed cotton yield was observed. Similar results were earlier recorded (Swami and Swami, 1986). The plant height in selected mutants were ranged from 135–145 cm, which was comparatively higher than untreated plants having a range of 125–140 cm. Mutant lines also showed higher number of bolls as compared to control. Earlier, (Joseph *et al.*, 2004) reported significant variations in plant height in cassava mutants.

As a requirement of mandatory evaluation in national and provincial coordinated trials, NIAB-2008 was evaluated in yield trials. The data were statistically analyzed by analysis of variance technique (Steel *et al*., 1997). Significant differences were recorded for seed cotton yield compared to standard varieties. In local trials (preliminary & advanced), it produced 32.5% higher seed cotton than standard CIM-496 (Table 3). These standards are decided at national level and are the most popular and dominating cultivars.

In PCCT, it produced 17.8% and 61.8% higher seed cotton yield than standard during two years testing. On an average in PCCT, it produced 32.7% higher seed cotton yield. In NCVT, it produced 58.2% and 11.7% more seed cotton yield than standard in Punjab and on country basis respectively. During second year testing it produced 6.2% and 6.4% higher seed cotton yield than standard MNH-786 in Punjab and at country basis respectively (Table 4).

Table 1: Developmental History of NIAB-2008

Parentage/Pedigree	Remarks
Cross attempted (NIAB-78 x REBA-288) with irradiated pollen @ 10Gy of gamma rays	Field conditions
$M_1 – M_5$	Field conditions
M_6	bulked
M_7 (M-07/09)	studied in strain test
NIAB-2008	PYT
-	AYT
-	PCCT
-	NCVT & 1.25 acre PSC trial
-	PCCT, NCVT &1.25 acre PSC trial
-	1.25 Acre trial at PSC, seed multiplication, spot examination by members of ESC
Expert subcommittee (ESC) of Punjab Seed Council (PSC) recommended. PSC approved in its 46th meeting in May, 2016	

Table-2: Comparison of selected mutant with parents for different traits

Variety/ Traits	Plant Height (cm)	CLCV rating	Bolls/ Plant	Boll weight (g)	Yield/ Plant (g)	GOT (%)	Staple Length (mm)	Fineness µg/inch	Strength		U.I (%)	Maturity (%)
									TPPSI	G/tex		
NIAB-78 (P)	140	3-4	68	3.0	200	36.60	27.3	4.50	93.0	27.5	84.0	84.0
REBA-288 (P)	160	0	30	3.0	90	36.50	27.4	4.90	92.6	27.0	-	-
NIAB-2008	145	0-3	70	3.6	230	37.83	31.16	4.74	92.2	27.55	83.5	80.5

Table 3: Average yield Performance of NIAB-2008 in various trials at NIAB

Name of trial		Place	Yield (kg ha^{-1})			% increase
			NIAB-2008	CIM-496	MNH-786	
Preliminary Yield Trials	1st year	NIAB, Faisalabad	4523	3582	-	26.3 %
	2nd year		4592	3423	-	34.1%
		Average	4558	3502	-	30.0 %
Advanced Yield Trials	1st year	NIAB, Faisalabad	4240	3251	-	30.4%
	2nd year		2662	1829	-	45.5%
		Average	3451	2540	-	35.9 %

Table 4: Average yield performance of NIAB-2008 in multi-location adaptability trials

Name of trial		Place	Yield (kg ha^{-1})			% increase
			NIAB-2008	CIM-496	MNH-786	
PCCT	1st year	Punjab	2663	2261	-	17.8 %
PCCT	2nd year	Punjab	1872	1157	-	61.8%
		Average	2268	1709	-	32.7 %
NCVT	1st year	Punjab	1993	1260	-	58.2 %
NCVT	2nd year	Punjab	1865	-	1756	6.2%
		Average	1929	1260	1756	32.2%
1.25 acre (PSC)	1st year	KWL	1715	1339	-	28.1%
1.25 acre (PSC)	2nd year	KWL	1698	-	-	-
1.25 acre (PSC)	3rd year	KWL	2117	-	-	-
		Average	1843	1339	-	37.6%
Average seed cotton yield (Tab 2 & 3)			2810	2070	1756	35.7%

% increase in yield over CIM-496 = 35.7%

The yield performance of candidate lines in PCCT and NCVT trials was compared using Fisher's least significant difference (LSD) procedure. All the varieties showed significant differences (Table 5). Similar results were earlier reported in cotton (Nepolean, 1999).

Pathological Studies

Screening against cotton leaf curl virus (CLCuV) disease for the selected mutants was continued after selection. The finally selected mutant NIAB-2008 was recorded resistant to CLCuV disease (old strain) like standards CIM-499 and CIM-496. The field response to different diseases of cotton in NCVT was analyzed (Table 6). NIAB-2008 showed stunting of 1.3% compared to GS-378 (1.4%) and VH-289 (1.7%). Whereas the boll rot analysis of NIAB-2008 showed the disease incidence of 2.8% compared to SLH-334 (3.1%), FH-4243 (3.5%), CIM-573 (2.4%), GS-321(5.6%), CRIS-486(4.7%), RH-625(3.0%) and MNH-786 (3.5%). The response of NIAB-2008 to CLCuV-B was also studied in NCVT. NIAB-2008 showed better performance against cotton leaf curl virus (CLCuV-B) compared to standard MNH-786. For grafting studies, ten pots each for each variety were sown in glass house.

Table 5: Least significant difference (LSD) comparison of candidate's varieties and standards in PCCT and NCVT

Variety	SCY (kg/ha) PCCT 2008-2009	Variety	SCY (kg/ha) PCCT 2010-2011	Variety	SCY (kg/ha) NCVT 2010-2011	Variety	SCY (kg/ha) NCVT 2010-2011
FH-942	3007 BCDEF	BH-172	1845 GHIJ	CRIS-486	2072 CDEF	FH-2015	1770 GHI
RH-620	2739 EFGHI	FH-114	2277 ABC	VH-289	2246 BC	VH-289	2262 BCDE
VH-255*	2995 CDEF	NIAB-2009	2490 BCDE	CIM-557	1753 EFGH	CIM-608	2158 BCDEF
CRSM-2007	3033 ABCDE	FH-4243 (ok)	1997 EFGH	NIA-78	16686 GHI	GH-114	1556 I
MG-6	2986 CDEF	MNH-814	2359 ABCD	BH-172	2192 BCD	BH-175	2073 BCDEFG
CIM-557	2553 HIJK	FH-113/326	2112 DEFGH	NIAB-852	2062 BCDE	NIAB-9811	2744 A
GS-1	2305 JKL	IR-NIAB-824	1522 JK	CIM-588	1564 HIJ	CRIS-486	1904 EFGHI
VH-277	2270 KL	VH-289	2125 DEFG	RH-514	1805 EFGH	SLH-334	2001 CDEFGH
NIAB-852	2996 6th CDEF	FH-207 Bt	1787 HIJ	NIAB-2008	1993 CDEF	FH-4243	1979 DEFGH
CRSM-38	2534 HIJK	MNH-886	1933 FGHI	PB-900	1764 FGHI	CIM-573	2343 BC
VH-207	2807 DEFGH	NIBGE-314	2181 CDEF	NN-3	2280 BC	GS-321	1654 HI
SLH-317	2608 HIJ	CIM-595	1366 KL	FH-942	1955 DEFG	GS-378	1657 HI
CIM-496(Std)	2261 KL	CIM-496 (St)	1157 L	CRSM-2007	1952 CDEF	NIAB-2009	2383 AB
GS-14	2558 HIJK	NIAB-2010	1633 IJK	SLH-317	2331 AB	NIBGE-314	2108 BCDEFG
CIM-554	2551 HIJK	VH-259	2517 AB	GS-27	1241 JK	MNH-814	2108 BCDEFG
PB-900	2202 L	CRSM-2007	2278 BCDE	MNH-814	2574 A	RH-625	2321 BCD
NIAB-777	2761 EFGHI	FH-113/128	2005 EFGH	CIM-573	2192 BC	MNH-786(St)	1756 GHI
SITARA-008*	2443 IJKL	NIAB-9811	2676 A	FH-941	1999 CDEF	CRIS-494	2048 BCDEFG
A-One*	3247 ABC	CIM-573	1175 L	GS-14	1464 IJK	NIAB-2008	1865 FGHI
FH-941	3226 AB	BH-175	1821 GHIJ	CIM-496(St)	1260 K	NIAB-2010	1962 DEFGH
BH-172	2691 FGHI	SLH-334	1945 FGHI				
FH-2015	2973 CDEFG	MNH-888	2382 ABCD				
NIAB-2008	2663 GHI	RH-826	1808 GHIJ				
FH-113*	3108 ABCD	NIAB-2008	1872 FGHI				
NN-3	2757 EFGHI						
Alseemi-hybrid	3335 A						
CV%	13.85		23.51		17.41		20.16

Fig 1: Mutant NIAB-2008(A) in comparison with parents NIAB-78 (B) and REBA-288 (C) with plant type, branching and fruiting pattern

Graft inoculation of the plants with CLCuV-B was done by following the bottle shoot grafting method (Akhtar *et al*., 2002, 2010). Results showed that number of days taken to appear the symptoms (after grafting) in case of NIAB-2008 were 14–20 compared to 12–18 of MNH-786 (Table 7).

Fibre Quality Analysis

The results of fibre quality testing studies revealed that fibre quality traits of NIAB-2008 are either better or comparable to standard. Fibre quality testing was carried out at four standard laboratories. On an average of all labs, NIAB-2008 scored fibre quality i.e., ginning outturn (GOT)

Table 6: Field Response of NIAB-2008 to different diseases in comparison to standard

NCVT Strain	Disease incidence (%age)		CLCV disease Index (%age)
	Stunting	Boll rot	
NIAB-2008	1.3	2.8	98.46
GS-378	1.4	3.3	98.48
VH-289	1.7	3.4	94.08
MNH-786 (St)	0.0	3.5	99.14
SLH-334	0.0	3.1	96.03
CIM-573	1.2	2.4	96.85
FH-4243	0.4	3.5	98.42
GS-321	2.1	5.6	99.25
CRIS-486	0.4	4.7	93.29
RH-625	0.0	3.0	98.81

Table 7: Screening of different strains against CLCV through petiole graft transmission technique at CCRI, Multan

Variety/Strain	No. of days taken to appear the symptoms (after grafting)
BH-175	13-18
SLH-334	15-24
FH-4243	12-16
GS-321	14-18
RH-625	14-20
MNH-786	12-18
NIAB-2008	14-20
GS-378	13-18
VH-289	13-25

Table 8: Fibre quality characteristics of NIAB-2008 compared to standard MNH-786 tested at four standard labs

Lab./Parameters	GOT (%)	Staple Length (mm)	Mic. µg/inch	Strength		U.I (%)	Maturity (%)
				Tppsi	g/tex		
CRI, Faisalabad	37.99	31.00	4.60	90.0	-	-	80.5
CCRI, Multan	-	30.70	4.95	94.3	-	83.7	-
NIBGE, Faisalabad	-	31.20	4.80	-	29.90	84.8	-
APTMA, Lahore	-	31.75	4.60	-	25.20	82.0	-
NIAB-2008 (Av.)	37.99	31.16	4.74	92.2	27.55	83.5	80.5
MNH-786 (St)	38.59	28.71	4.86	103.2	31.5	82.1	82.5

Table 9: Salient morphological and plant characteristics of NIAB-2008

Plant character	Range	Plant character	Range
Days to maturity	130-150 days	Flower Characteristics	
Days to opening	120-125 (50%)	Days to flowering	45-55 days (50% flowering)
Seedling Characteristics		Flowering duration	Medium
Seedling length	6.2-7.3cm	Boll Characteristics:	
Plant Characteristics:		Boll length (cm)	3.9- 4.6cm
Plant height	120-145 cm	Boll breadth (cm)	2.3-2.8
Nodes to 1st Monopodia	7-8	Boll/plant	60-70
Monopodial/plant	0-3	Boll opening	Good
Sympodia/plant	20-28	Boll weight	3.3-3.6 gram
Leaf Characteristics:		Seed Characteristics:	
Leaf length	15-17cm	Seed length (mm)	7.0-7.5 mm
Leaf width	16-18 cm	Seed width (mm)	4.1-4.7 mm
Leaf nectaries	Present	Seed index (g)	8.0-8.7g
Leaf hairiness	Medium	Best Sowing time:	15st April to 30th May

percentage of 37.99%, fibre length of 31.16 mm (fall in long staple category), fibre fineness of 4.74 µg/inch, uniformity index of 83.5%, maturity 80.5%, fibre strength of 92.2 TPPSI (Table 8). It has better fibre quality traits as compared to both parents. Ginning out turn percentage, staple length, fineness, strength etc has been improved through irradiation treatment as compared to control parents. Similar observations were earlier reported (Ibragimov *et al.,* 1965) and significant variation between individual tetraploid lines for fiber contents was observed (Smith *et al.*, 2004).

Various important morphological, plant, leaf, flower, boll and seed characters are given in Table 9. It is maintained regularly at NIAB and breeder nucleus seed (BNS) is being provided to farmers/seed producing agencies.

Discussion

This study provides an extensive research on the induced mutations through (γ) gamma rays used on pollen grains (germ cell) in cotton and variations in succeeding generations. The results showed that the low doses of gamma rays are useful to create the desired variation in the mutated populations. The overall changes recorded in the selected mutant line are may be due to genetic variation caused by gamma irradiation. In some earlier findings such type of reports i.e. variations in leaf shape (Muthusamy and Jayabalan, 2000), in term of increase in yield (Muthusamy *et al*., 2005), twin boll and boll shape irregularities (Muthusamy *et al.*, 2004) and other morphological variations (Muthusamy and Jayabalan, 2001) was observed in cotton mutant lines.

The result of NIAB-2008 has showed that, pollen irradiation of suitable parent before cross pollination is an appropriate technique to create useful variability in cotton. By suing seed irradiation method, a large M_2 population is required because the whole genome is irradiated and whole genetic makeup is disturbed (Iqbal, *et al.,* 1994). Whereas in case of pollen irradiation half of the genome receives the irradiation, hence major changes are less compared to seed irradiation (Aslam, 2000; Aslam *et al*., 2009). These results support the effectiveness of pollen irradiation technique to improve crop plants.

In pollen irradiation technique, male pollen is irradiated at low doses of gamma rays before cross pollination and therefore more recombinations are brought as compared to seed irradiation, simple cross breeding and backcrossing (Aslam *et al*., 2009). Due to this a small M_2 population is required in case of pollen irradiation because of higher rate of mutations/recombination's (Wang, 1990).

In this study, lower dose of gamma irradiation showed enhancing effects on growth of vegetative and reproductive parts of plants along with yield and yield contributing characters. Such type of enhancement is due to increase in enzymes activity, which is required in biosynthesis of hormone in the cell (Vagera *et al.*, 1976; Yue and Zou, 2012), which ultimately increases the growth and number of cells and the whole plant. Induction of mutations through this comparatively easier method was earlier reported by different researchers (Aslam *et, al.*, 1994; Aslam and Ealahi,

2002) and is in accordance with our present finding in cotton.

Due to irradiation effects, NIAB-2008 on an average exhibited 35.7% higher yield than standard in NCVT and PCCT. NIAB-2008 being moderately hairy with medium sized erect plant type with short to medium short sympodia is suitable to obtain good yield. It has better leaf foliage which is suitable for high density planting. Its plant type facilitates the easy application of pesticides to control the cotton insect pests. Its fibre quality characters are according to prescribed standard and as per requirement of textile sector, which is the dire need of national production and good quality cotton for meeting the domestic textile industry requirements.

Due to these useful characters, the mutant NIAB-2008 was recommended and approved by Punjab Seed Council (PSC) in its 46th meeting in May, 2016. It is being recommended to farmers' community for general cultivation in Punjab province of Pakistan. Its cultivation will be adding to the national exchequer through export of raw cotton and value added products and to meet the demand at national and world level as well. Being a long staple variety, it will have very positive implications of obtaining additional returns at national economy to meet the demand of long staple cotton at national as well as at international level.

Conclusion

With substantial varietal difference, the pollen irradiation technique can be effectively used to produce high seed cotton yield with fiber of long staple. Further studies are needed to find the possible mechanism(s) of induced mutations at molecular level that cause improvement as noted in the present study.

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