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NIAB-852: A NEW HIGH YIELDING AND BETTER FIBRE QUALITY COTTON MUTANT DEVELOPED THROUGH POLLEN IRRADIATION TECHNIQUE

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Abstract

The new high yielding and better quality cotton mutant NIAB-852 was developed through pollen irradiation technique. This paper describers about the development of cotton mutant through induction of mutations and its evaluation. A cross between NIAB-78 (local variety) and REBA-288 (exotic line) using gamma irradiated male pollen @ 10 Gray (Gy) of gamma rays before cross pollination was attempted. The objective was to create new genetic variability and select the desirable new cotton mutants. After irradiation followed by hybridization subsequent generations were raised to investigate the effect of irradiation treatment. Significant variations from control/parents were observed. From M_1 generation, the M_2 population was grown and different desirable mutants having higher yield, early maturity, resistance/tolerance to diseases were selected. These were evaluated for yield potential and desirable other economic traits in different generations till uniformity were achieved. From selected mutants, an elite mutant i.e. NIAB-852 was finally selected for further evaluation. It has a suitable plant type, better leaf foliage, moderately hairy, early maturing and higher yield potential with acceptable fiber quality traits. It was evaluated for seed cotton yield, adaptability, resistance/tolerance to diseases, and fibre quality in different trials. It produced 16.4% higher seed cotton yield as compared to standard variety CIM-496 in local trials at NIAB. It also produced higher seed cotton yield in regional (23.4%), provincial (18.6%) and national (26.8%) coordinated adaptability trials compared with standard. On an average of all trials, NIAB-852 produced seed cotton yield of 3153 kg.ha^{-1} compared to standard CIM-496 (2674 kg.ha^{-1}) producing 18.0% higher seed cotton yield. It also performed better than standards in different regions/provinces by producing 4% to 17% higher seed cotton yield. The mutant NIAB-852 has desirable fibre quality traits i.e. GOT 38.8 %, fibre length 30.1 mm (long staple category), fibre fineness 4.68 µg/inch, uniformity index 83.1%, fibre maturity 81.1% and fibre strength 94.3 TPPSI. It has characteristics of good boll bearing, rapid boll formation at later stages and better tolerance to cotton leaf curl virus (CLCuV) disease and insect's pests due to early maturity. From these results it is concluded that low dose pollen irradiation technique in cotton has effectively stimulate/increase the agronomical characters and tolerance to disease.

Key words: Mutant, Cotton, Pollen irradiation, Yield, Fibre quality, Long staple, NIAB-852.

Introduction

Cotton is a crop of global importance and Pakistan is one of the prominent cotton producing and consuming country of the world. It is an important cash crop and provides the basis for a national textile industry and contributes a major share in foreign exchange earnings. All most all parts of cotton plants are used extensively but it is mainly cultivated for its fibre and seed oil (Pandey, 1998). It is cultivated on an area of 3125, 000 ha with an annual production of 12.8 million bales (Anonymous, 2013-14) which is less as per our requirement. Millions of people in Pakistan are linked with cotton cultivation, ginning, oil industries, trade and spinning processes. However, cotton producers in Pakistan are currently faced with rising production costs and static return (Haidar *et al*., 2007).

Lot of efforts has been made by cotton researchers to develop cotton varieties having high yield potential, desirable fibre quality and tolerance/resistance to insect pests and diseases through conventional breeding approaches. However, there are limitations of availability of sufficient genetic variability in the native germplasm (Haidar *et al*., 2012). The success of all conventional breeding approaches is highly correlated with the genetic variability present within the existing germplasm. Numbers of approaches are being adopted in conventional breeding linked with advanced techniques to create new genetic variability in cotton. Mutations techniques have been used in different crops to improve yield, quality, disease and pest resistance and produced additional germplasm with desired traits (Maluszynski *et al*., 1995). Number of mutants of different crop plants have been developed and released in different countries of the world with improvement in some characters (Ahloowaila *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), *Chrysanthemum* (Datta *et al.,* 2004), groundnut (Muthusamy *et al.,* 2007) and rapeseed (Xiang *et al*., 2016).

The approaches like; the exposure of seed to ionizing radiations and the treatment of pollen with low doses of gamma rays before cross-pollination resulted in creating genetic variability in different crop species. In cotton, Carnelius, 1973, developed a new cotton variety MCU-7 through seed irradiation technique. Jalil Miah and Yamaguchi, 1965, reported increase in genetic variability for different quantitative traits in segregating F_2/M_2 population in rice. Micke *et al*., 1987 also reported seed irradiation studies in various crops i.e. wheat, barley, rice, maize etc in different countries. Iqbal *et al*., 1991, develop different mutants of cotton by using seed irradiation and F_1 seed irradiation techniques. Another technique i.e. treatment of pollen with low doses of gamma rays before cross-pollination is also utilized by different researchers to create genetic variability in different crop species (Pate & Duncan, 1963; Vig, 1973; Krishnaswami & Kothandaraman, 1976; Aslam & Stelly, 1994; Aslam, 2000). Mohajer *et al*., 2015, observed improvement in nutritional quality of important forage crop Sainfoin. Whereas, Baig *et al*., 2015, reported floral diversification and diversity in color pattern in Rosa Spp after gamma irradiation treatments.

In the present study, due to induction of mutations through pollen irradiation, the selected mutant exhibited significantly higher seed cotton yield than standard in multi-location regional/zonal trials. The mutant also exhibited good boll bearing, rapid boll formation at later stages and better tolerance to cotton leaf curl virus disease and insect's pests due to early maturity. The selected mutant showed medium leaf size and foliage which is suitable for high density planting. Its fibre quality characters are according to prescribed standard and better than the parents utilized for irradiation/hybridization. Earlier, Sanamian, 2003, reported various genomic and chromosomal mutations in M_2 families developed through pollen irradiation. He concluded that variability in cotton plants treated with pollen irradiation is mainly because of chromosomal rearrangements and genomic mutations during meiosis.

The main objectives of the present studies were aimed at to create genetic variability through crosses with irradiated male parent pollen, selecting the desirable mutants from the segregating populations. Their evaluation for high yield potential, desirable fibre quality and wider adaptability in the cotton growing areas for confirmation of stability and adaptability and finally recommend to farmers for its cultivation. The manuscript details the report of induction of mutations in cotton using low doses of gamma irradiation on germ cells and selection and evaluation of desirable cotton mutant.

Materials and Methods

Plant material: Selfed seeds of commercially approved cotton variety NIAB-78 and an exotic line REBA-288 were planted at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan and approximately sixty plants of each were developed. The local variety NIAB-78 has good agronomic characters 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. 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. The cross was made by utilizing NIAB-78 as female parent with REBA-288 as male parent.

Radiation treatment: Male parent pollen was collected from covered flowers after anthesis and irradiated with gamma rays @10 Gy of gamma rays before cross-pollinations from ^{60}Co irradiation source. The irradiation was performed at room temperature at NIAB, Faisalabad, Pakistan. 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 seed along with both parents as control at experimental field of NIAB, Faisalabad. The seeds were planted at a spacing of 30 cm plant to plant and 75 cm row to row distance. Seeds were collected from the bolls of M_1 generation plants and their characteristics were recorded along with control. 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. In M_1 generation, groups of plants were selected and carried forward on bulk basis. While in advanced generations (M_2, M_3, M_4) single plants were selected. Thirty selected mutants were planted in M_3 generation in replicated plant progeny test (RPT) with three replications. These progenies were studied in M_3 generation for their breeding behavior and economic traits and one higher yielding progeny was selected. Plant progeny rows were also studies in M_4 generation to confirm its higher yield potential and other desirable traits and to see its breeding behavior/uniformity. Finally the progeny M-7/03 was selected from M_6 and bulked for evaluation under the name of NIAB-852. All these generations were raised and evaluated at NIAB where the soil type is clay loam. Required agronomic practices and plant protection measures were carried out throughout the crop growing season to control or minimize the sucking and bollworm insect pests.

Evaluation in adaptability trials: Various trials (local yield trials, zonal yield trials i.e. National Coordinated Varietal Trials (NCVT), Provincial Coordinated Cotton Trials (PCCT), 1.25 acre Punjab Seed Corporation (PSC) farm trials etc.) were conducted at NIAB, at farmer's fields, public sector experimental institutes etc. The objective was to analyze yield potential, fibre quality traits and wider adaptability in different climatic zones of Pakistan. Related studies i.e. earliness studies were also carried out at NIAB.

Screening for disease and insect pests: Screening against cotton leaf curl virus (CLCuV) disease was done through grafting and whitefly inoculation technique. For grafting studies, ten pots for each variety were sown in glass house. Plants were infected/inoculated with CLCuV disease through grafting by following the bottle shoot grafting method as described by Akhtar *et al*. (2002, 2010) in earlier generations. Whereas, most susceptible spreader lines were planted in between the rows to serve as spreader/source of inoculum. Entomological studies regarding its response to sucking pests and damage by bollworms were also conducted under optimized spray condition at NIAB Faisalabad.

Fibre characters analysis: Fibre quality characters of mutant lines along with the control 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-852 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 HVI.

Statistical analysis: The experiments related to yield evaluation were 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 analyzed by using analysis of variance (ANOVA) as earlier described by Steel and Torre, 1980. In addition data for seed cotton yield in various adaptability trials were compared using Fisher's least significant difference (LSD) procedure.

Results

In this study, a local cotton variety NIAB-78 was crossed with an exotic line REBA-288 by using gamma irradiated pollen to induce variation leading to development of mutants with good yield and fibre characters. The developmental history of selected mutant NIAB-852 is given in Table 1. Data on different traits on selected mutant along with control lines is given in detail and discussed.

Influence of mutagenic treatments on selected plants: Significant differences were observed between selected mutants compared to the control plants. There was maximum variation in M_2 generation and plants possessing desirable traits were selected. Succeeding generation M_3-M_6 was evaluated, and as a result M-07/03 with high yield potential was selected which was later named as NIAB-852. The mutants/plants developed from irradiated pollen showed different growth features during early seedling as well as normal plants development in the field conditions. Similar results were earlier observed in cotton by Muthusamy & Jayablan; 2000, 2001.

Field evaluation of mutant lines: A range of morphological differences was recorded from seedling stage to maturity in M_2, M_3 and M_4 generations. Morphological as well as yield characters along with fibre traits i.e. ginning out turn percentage (GOT%), fibre fineness, staple length, fibre strength, uniformity ratio percentage were recorded and analyze the effect of irradiation treatments in comparison with the control (parents etc). NIAB-852 showed moderately hairy, semi-compact, sympodial type (20-30) with fruit bearing monopodia (0-3). It has desirable leaf foliage and medium plant stature (130-150cm). It matures in 160-170 days with high fruiting load to give high yield. The seeds of NIAB-852 are bold and fuzzy with dusky white fuzz. It has morphological traits which are economically beneficial for its cultivation as crop (Table 2). The flowering periods of mutants were decreased than parents and showed increase in yield. It showed earliness and better seed cotton yield as compared to control. Similar results were earlier recorded by Swami & Swami, 1986. In cassava significant variations in mutant lines for plant height were earlier reported by Joseph *et al.* (2004).

As per requirement of mandatory evaluation of finally selected mutant in various provincial and national coordinated trials for its release as commercial variety and to check its adaptability. It was evaluated in various trails. It produced 16.4 % higher seed cotton yield as compared to standard CIM-496 in local trials at NIAB. Whereas in regional adaptability trials in different regions of Punjab province of Pakistan (Sahiwal, Multan, Bahawalpur, Khanewal and Vehari). NIAB-852 produced 23.4% higher yield compared to standard CIM-496. These standards are decided at national level and are the most popular and dominating cultivars. In Provincial Coordinated Cotton Trials (PCCT), it produced 4.7% to 32.5% higher seed cotton yield than standard. On an average of two years in PCCT, it produced 18.6% higher seed cotton yield than CIM-496 (Table 3) and performed better at Multan, Khanewal, Faisalabad and Bahawalpur regions of Punjab province (Table 4).

Table 1. Developmental History of NIAB-852 (1998-2016).

Parentage/Pedigree	Activities/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 (M-7/03)	bulked
M_7 (NIAB-852)	studied in strain test
M_8	Preliminary Yield Trial (PYT)
-	Advanced Yield Trial (AYT)
-	PCCT
-	PCCT, NCVT & 1.25 acre PSC trial
-	NCVT &1.25 acre PSC trial
-	1.25 Acre trial at PSC, spot examination by members of ESC

Recommendation by Expert subcommittee (ESC), Approval of Punjab Seed Council (PSC) for general cultivation in Punjab

Provision of BNS to farmers and maintenance at NIAB

Table 2. Salient plant characteristics of NIAB-852 at different stages of plant growth.

Characters	Range	Characters	Range
General:		**Boll Characteristics:**	
Days to maturity	160-170 days	Boll bearing habit	Cluster
Days to opening	125-130 (50%)	Boll shape	Round
Seedling characteristics:		Boll length (cm)	3.8- 4.6cm
Seedling length	6.3-7.4cm	Boll breadth (cm)	2.2-2.8
Plant characteristics:		Peduncle length	1-1.5cm
Plant height	130-150 cm	Boll/plant	60-75
Monopodial/plant	0-3	Boll opening	Good
Sympodia/plant	20-30	Boll weight	3.0-3.5 gram
Leaf characteristics:		**Seed Characteristics:**	
Leaf color	Light green	Seed length (mm)	7.0-7.9 mm
Leaf length	16-18cm	Seed width (mm)	4.2-4.8 mm
Leaf width	7-9 cm	Seed index (g)	8.0-9.0g
Flower characteristics:		Seed coat color	Brown
Days to flowering	70-80 days (50% flowering)	Seed Fuzz	Fuzzy
Flowering duration	Short	Fuzz color	Dusky white

Table 3. Yield performance of NIAB-852 compared with standard in adaptability trials.

Name of trial	Place	Yield (kg.ha^{-1})		% increase over check
		NIAB-852	CIM-496	
Micro Varietal trials	NIAB, Faisalabad	5126	4416	16.1
Macro Varietal trials		4583	3923	16.7
	Average	**4854**	**4170**	**16.4**
Regional Adaptability trials in Punjab	i) CRS, Sahiwal	3140	2834	10.8
	ii) CCRI, Multan	2098	1453	44.4
	iii) RARI, Bahwalpur	2770	2286	21.2
	iv) PSC, Khanewal	3485	3371	3.4
	v) CRS,Vehari	1885	892	52.7
	Average	**2676**	**2168**	**23.4**
PCCT (1st year, 2007-08)	Punjab	2358	2253	4.7%
PCCT (2nd year, 2008-09)	Punjab	2996	2261	32.5%
	Average	**2677**	**2257**	**18.6%**
NCVT(1st year, 2008-09)	Punjab	3065	2940	4.3%
NCVT(2nd year, 2009-10)	Punjab	2062	1260	63.4%
	Average	**2562**	**2100**	**26.8%**
Average seed cotton yield of NIAB-852 and CIM-496		**3153**	**2674**	**18%**

% Increase in yield of NIAB-852 over CIM-496 = 18%

Table 4. Comparative yield of NIAB-852 in PCCT under different agro climatic zones in Punjab province.

Location	1st Year Yield (kg. ha^{-1})		± Over CIM-496 (%)	Location	2nd Year Yield (kg. ha^{-1})		± Over CIM-496 (%)
	NIAB-852	CIM-496			NIAB-852	CIM-496	
CRI, Faisalabad	2332	1794	29.99	CCRI Multan	3038	1350	125.04
CRS, Multan	2750	2604	5.61	CRS, Multan	2106	780	170.0
CRS Vehari	1094	700	56.29	PSC, Khanewal	3856	3265	18.10
CRI, R. Y. Khan	2330	2099	11.01	ARS, Bahawalpur	2979	2946	1.15
CRS , Sahiwal	2677	2597	3.08	CRS, Bahawalpur	3498	2493	40.1
RSS, Jhang	1934	1545	25.18	CRS, Vehari	2529	1991	30.67
AA. Farm, Multan	1989	1932	2.95	CRS, Sahiwal	2897	2173	33.32
CRI, Faisalabad	1018	1167	-12.77	RSS, Jhang	3825	2929	30.59
ARS, Bahawalpur	2698	2698	0.00	CRI, R.Y.Khan	2024	1378	46.88
ARS, Khanewal	2247	2543	-11.64	C RI, Faisalabad	2229	2581	-13.64
ARS, Karor	2503	2583	-3.10	NIAB, Faisalabad	3972	2985	33.07
CCRI, Multan	3461	3210	0.08				
NIAB Faisalabad	3732	3288	13.50				
CRS, Piplan	2253	2789	-19.22				
Average	**2358**	**2253**	**4.66**		**2996**	**2261**	**32.49**

Table 5. Comparative yield of NIAB-852 in NCVT under different agro climatic zones in Punjab province.

Location	1st Year Yield (kg.ha^{-1})		± Over CIM-496 (%)	2nd Year Yield (kg.ha^{-1})		± Over CIM-496 (%)
	NIAB-852	CIM-496		NIAB-852	CIM-496	
NIAB Faisalabad	4134	4241	-2.52	2864	2412	18.74
CRS, Sahiwal	3384	2738	23.59	1475	440	235.23
CRI, Faisalabad	1315	2292	-42.63	1666	1708	-2.46
CCRI Multan	3262	2138	52.57	2670	1695	57.52
CRS, Multan	1445	1146	26.09	1377	718	91.78
CRS, Vehari	2751	2456	12.01	2409	774	211.24
PSC, Khanewal	3632	3578	1.51	1527	633	141.23
CRS, Bahawalpur	2980	3135	-4.94	2261	1372	64.80
CRS, R.Y. Khan	1420	1750	-18.86	1772	961	84.39
Average	**3065**	**2940**	**4.3**	**2062**	**1260**	**63.4**

Table 6. Yield performance and significance of candidate's varieties and standard varieties in PCCT and NCVT.

Seed cotton yield (PCCT) Kg hac^{-1}				Seed cotton yield (NCVT) Kg hac^{-1}			
Variety	**1st Year**	**Variety**	**2nd Year**	**Variety**	**1st Year**	**Variety**	**2nd Year**
VH-255	3054 A	FH-942	3007 BCDEF	GH-102	2584 DE	CRIS-486	2072 CDEF
FH-942	2711 AB	RH-620	2739 EFGHI	PB-900	2945 BCD	VH-289	2246 BC
NIAB-846	2650 BC	VH-255	2995 CDEF	SLH-317	2985 ABC	CIM-557	1753 EFGH
MG-3	2601 BCD	CRSM-2007	3033 ABCDE	NIAB-852	3065 BCD	NIA-78	16686 GHI
FH-113	2569 BCDE	MG-6	2986 CDEF	CRIS-129	3190 ABC	BH-172	2192 BCD
CRSM-70	2542 BCDE	CIM-557	2553 HIJK	CRSM-38	2876 BCD	NIAB-852	2062 BCDE
NIBGE115	2537 BCDE	GS-1	2305 JKL	FH-942	2876 BCD	CIM-588	1564 HIJ
NIAB-777	2501 BCDE	VH-277	2270 KL	CIM-554	3095 ABC	RH-514	1805 EFGH
NIAB-852	2358 BCDEF	NIAB-852	2996 CDEF	NIAB-777	3197 ABC	NIAB-2008	1993 CDEF
SLH-284	2339 BCDEF	CRSM-38	2534 HIJK	TH-06/2	2353 E	PB-900	1764 FGHI
CIM-554	2325 CDEF	VH-207	2807 DEFGH	NN-3	2956 BCD	NN-3	2280 BC
BH-168	2302 CDEF	SLH-317	2608 HIJ	NIA-78	2259 E	FH-942	1955 DEFG
GS-1	2273 DEF	CIM-496(S)	2261 KL	BH-172	3363 A	CRSM-2007	1952 CDEF
RH-610	2264 DEF	GS-14	2558 HIJK	CRSM2007	2802 CD	SLH-317	2331 AB
CRSM-38	2257 DEF	CIM-554	2551 HIJK	FH-941	3268 AB	GS-27	1241 JK
CIM-496 (S)	2253 DEF	PB-900	2202 L	GS-1	2646 DE	MNH-814	2574 A
FH-941	2251 DEF	NIAB-777	2761 EFGHI	CIM-557	3135 ABC	CIM-573	2192 BC
BH-167	2248 DEF	SITARA-008	2443 IJKL	VH-278	2510 E	FH-941	1999 CDEF
RH-541	2198 EF	A-One	3247 ABC	GS-14	2952 BCD	GS-14	1464 IJK
ASR-1	2108 F	FH-941	3226 AB	CIM-496(S)	2940 CD	CIM-496(S)	1260 K
VH-260	2025 FG	BH-172	2691 FGHI				
CIM-541	1703 GH	FH-2015	2973 CDEFG				
MG-2	1665 GH	NIAB-2008	2663 GHI				
MG-1	1537 H	FH-113	3108 ABCD				
		NN-3	2757 EFGHI				
		Alseemi-hyb	3335 A				
CV%	21.78		13.87		14.79		17.41

In National Coordinated Varietal Trials (NCVT), at Punjab locations it produced 4.3% to 63.4% higher seed cotton yield compared with CIM-496. On an average in NCVT, it produced 26.8% higher seed cotton yield (Table 3) and performed better at Faisalabad, Khanewal, Multan and Bahawalpur regions (Table 5). On an average of all trials, NIAB-852 produced seed cotton yield of 3153 kg.ha^{-1} compared to standard variety CIM-496 (2674 kg. ha^{-1}) showing 18.0% higher seed cotton yield. The data for seed cotton yield in various adaptability trials (PCCT, NCVT) showed significant differences through LSD test (Table 6). From these results it is observed than NIAB-852 not only produced higher number of bolls but also produced significantly higher yield in comparison with the standard and parents. Similar results were earlier reported in cotton (Nepolean, 1999). NIAB-852 also showed good performance in different provinces of Pakistan. It produced 3.0% to 17.0% higher seed cotton yield compared to standard in different provinces/zones of Pakistan (Table 7). The standard CIM-496 was allocated at national level due to its good yield performance and it covers 20-30% of acreage in Punjab province which contributes more that 80% of acreage as well production of the country. Whereas, CRIS-134 standard were allocated for Sindh and Baluchistan provinces.

Table 7. Yield performance (kg ha^{-1}) of NIAB-852 in NCVT conducted in different provinces/zones in Pakistan.

Zone/ variety	1st Year		2nd Year	
	NIAB-852	CIM-496/Cris-134*	NIAB-852	CIM-496/Cris-134*
Punjab (Av)	3065	2940	2062	1260
KPK (Av)	1683	1320	1743	1487
Sindh* (Av)	2462	2623	3278	3603
Baluchistan* (Av)	1843	1738	-	-
Pakistan (Av)	2818	2737 (3% increase over standard)	2473	2113 (17% increase over standard)

Table 8. Earliness studies/Morphological traits of NIAB-852 recorded at NIAB.

Characteristics	1st year study		2nd year study	
	NIAB-852	CIM-496	NIAB-852	CIM-496
Plant height (cm)	142-160	147-158	136-160	149-157
No. of sympodia/plant	30-36	22-28	30-37	23-29
First boll retention at node number	6-7	7-8	6-7	7-8
Total number of fruiting positions	267	208	266	210
Total number of shedding points	155	132	153	134
Total number of boll retention	112	76	113	76
Percentage of shedding points	58.1	63.4	57.5	63.8
Number of days taken to maturity	136-151	140-155	139-159	145-160
Seed cotton yield (1st pick %age)	82.7	80	83.0	81

Earliness studies: Different morphological traits i.e. plant height, sympodia/plant, first boll retention at node number, total fruiting points, shedding points etc, were recorded to evaluate earliness of NIAB-852 compared to standard CIM-496. The results showed that the sympodia/plant of NIAB-852 is higher than CIM-496 which confirmed higher number of bolls/plant. Moreover, the numbers of days taken towards maturity of NIAB-852 are also less as compared to CIM-496. The same has been confirmed by higher percentage of seed cotton in 1st pick (82.7 %) than CIM-496 (80%). The more number of sympodia/plant and higher number of fruiting positions/boll retention of NIAB-852 confirmed more number of bolls/plant than CIM-496. It matures earlier than standard CIM-496 (Table 8). Similar results of early flowering mutants in cotton by mutagenic treatment were earlier reported by Swami and Swami, 1986.

Pathological studies: Screening against CLCuV disease for the selected mutants was continued after selection. The finally selected mutant NIAB-852 was recorded resistant to CLCuV disease (old strain). The response against presently prevailing strain i.e. cotton leaf curl virus-Burewala strain (CLCuV-B) disease was also studied. The most susceptible spreader lines were also planted in between the rows to serve as spreader/source of inoculum. In PCCT, observations showed that all the varieties showed varying disease intensity i.e. 16.67 to 100%. NIAB-852 with disease index of 38.67% showed better tolerance to CLCuV-B than CIM-496 having 53.63% disease indexe. The response of NIAB-852 to CLCuV-B was also studied in natural field conditions in NCVT. NIAB-852 with disease index 23.90% showed better tolerance to CLCuV-B as compared to CIM-496 with disease index of 46.53% (Table 9).

Entomological studies: Studies on NIAB-852 regarding its response to sucking pests and damage by bollworms were conducted at NIAB in NCVT. NIAB-852 showed less population of sucking i.e. whitefly (4.78/leaf), jassid (0.32/leaf) and bollworms (12.18%) compared to standard CIM-496 having higher population of sucking i.e. whitefly (4.93/leaf), jassid (0.31/leaf) and bollworms (12.57%) (Table 10)

Fibre quality analysis: The results of fiber testing studies revealed that fibre quality traits of NIAB-852 are either better or comparable to standard cotton varieties. Fibre quality of NIAB-852 was analyzed from four standard laboratories (CRI, Faisalabad, NIBGE, Faisalabad APTAMA, Lahore and CCRI, Multan) from the samples collected during spot examination by members of expert's sub-committee of PSC. During first year, the average fibre quality parameters recorded from samples collected from PSC farm were i.e. GOT (39.68), Fineness (4.84 µg/inch), fibre length (28.64 mm), fibre strength (97.3 TPPSI, 29.25 g/tex), uniformity index (82.3%) and fibre maturity (82.7%), Whereas during 2nd year testing, its average fibre quality parameters recorded were i.e. GOT (37.83), Fineness (4.45 µg/inch), fibre length (31.59 mm), fibre strength (91.2 TPPSI, 27.0 g/tex), uniformity index (83.0%) and fibre maturity (80.0%). On an average of two years fibre reports of four standard labs, NIAB 852 has fibre quality parameters i.e. GOT (38.8), Fineness (4.68 µg/inch), fibre length (30.1 mm), fibre strength (94.3 TPPSI, 28.13 g/tex), uniformity index (83.1%) and fibre maturity (81.3 %)(Table 11). All these fibre qualities are either better or at par with commercial standards. Ginning out turn percentage, staple length, fineness, strength etc has been improved through irradiation treatment as compared to control parents. These results were confirmed by similar finding earlier reported by Ibragimov *et al.,* 1965. In addition in terms of fibre contents significant differences between single lines of tetraplod cotton were observed by Smith *et al*., 2004.

Due to its good performance it was approved by Punjab Seed council for general cultivation by the farmers community. Maintenance of NIAB-852 is in progress and single plant progeny rows and bulks are being maintained by evaluating their true to type morphological traits and fibre quality. True to type seed production and provision to the farmers are also in progress (Table 12).

Table 9. Field response of different candidate varieties against cotton leaf curl virus disease (CLCuV-B) at NIAB.

PCCT				NCVT			
Varieties	**% Disease index**	**Varieties**	**% Disease index**	**Varieties**	**% Disease index**	**Varieties**	**% Disease index**
FH-113	74.26	BH-168	56.68	FH-942	27.89	NN-3	18.56
BH-167	93.16	MG-3	83.92	CRSM-38	24.21	CIM-496(S)	46.53
NIAB-777	73.58	CIM-541	91.69	CIM-557	25.68	BH-172	21.88
CRSM-38	100	NIAB-846	16.67	CRIS-129	28.49	PB-900	29.71
SLH-284	62.51	MG-2	91.69	GH-102	51.97	GS-14	43.10
ASR-1	38.90	VH-255	44.06	NIA-78	45.05	NIAB-777	32.19
RH-160	69.15	RH-541	36.12	NIAB-852	23.90	SLH-317	29.95
CIM-554	28.90	NIBGE-115	28.71	TH-06/2	59.17	GS-1	28.73
MG-1	54.61	FH-942	50.01	CRSM-2007	16.79	VH-278	68.50
NIAB-852	38.67	VH-260	94.69	CIM-554	32.29	FH-941	57.74
CRSM-70	39.59	CIM-496(S)	53.63				
GS-1	44.45	FH-941	64.30				

Table 10. Population of sucking insect pests, bollworms infestation of candidate varieties in NCVT.

Varieties	Sucking insects/leaf		Bollworms infestation (%)		Mean damage (% age Sq +Bolls)
	Whitefly	Jassid	Squares	Bolls	
FH-942	4.87	0.32	13.93	9.28	11.61
CRSM-38	4.79	0.29	13.88	13.33	13.61
CIM-557	4.78	0.29	14.53	11.08	12.81
CRIS-129	4.90	0.29	14.61	11.66	13.14
GH-102	5.06	0.37	15.21	10.68	12.90
NIA-78	5.02	0.27	13.19	10.34	11.77
NIAB-852	4.78	0.32	13.81	10.55	12.18
TH-06/2	4.66	0.26	15.34	11.94	13.64
CRSM-2007	4.98	0.28	14.00	10.01	12.01
CIM-554	4.58	0.27	17.04	10.55	13.80
NN-3	4.75	0.34	16.54	10.31	13.43
CIM-496 (st)	4.93	0.31	14.90	10.23	12.57
BH-172	4.78	0.29	13.58	10.54	12.06
PB-900	4.84	0.28	16.00	11.11	13.56
GS-14	4.55	0.32	16.75	11.53	14.14
NIAB-777	4.78	0.28	12.87	11.40	12.14
SLH-317	4.80	0.28	15.46	10.94	13.20
GS-1	5.18	0.30	15.00	11.86	13.43
VH-278	4.53	0.29	14.67	12.50	13.59
FH-941	5.26	0.32	15.53	9.93	12.73

Table 11. Fibre quality characteristics of NIAB-852 tested at four standard labs.

Character Lab./Year	GOT (%)		Staple length (mm)		Mic. (µg/inch)		Strength				U.I (%)		Maturity (%)	
							TPPSI		G/tex					
	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
CRI, Faisalabad	39.68	37.83	28.8	31.0	4.80	4.50	97.5	90.2	-	-	-	-	82.3	-
CCRI, Multan	-	-	28.8	30.6	4.90	4.45	97.7	92.1	-	-	-	-	85.6	-
NIBGE, Faisalabad	-	-	29.23	31.50	4.84	4.46	-	-	32.7	27.66	83.0	83.0	82.7	-
APTMA, Lahore	-	-	28.96	33.27	4.80	4.40	-	-	26.70	26.4	-	-	80.4	80
Labs Average	39.68	37.83	28.64	31.59	4.84	4.45	97.3	91.2	29.25	27.0	82.3	83.0	82.7	80
Two years average	**38.8**		**30.1**		**4.68**		**94.3**		**28.13**		**83.1**		**81.3**	

Table 12. Maintenance and provision of Breeder Nucleus seed (BNS) since approval of NIAB-852.

Variety	BNS provided to cotton growers/seed distribution agencies (Kg)				
	2011-12	2012-13	2013-14	2014-15	2015-16
NIAB-852	319	534	394	51.0	60

Fiber quality characters evaluated during 2015

Progeny bulks of NIAB-852	No. of bolls/ plant	GOT (%)	Fibre Fineness (µg/inch)	Fibre Strength (g/Tex)	Fibre length (mm)	Uniformity ratio (%)
NIAB-852-5	82	40.3	4.5	35.1	30.0	85.5
NIAB-852-7	56	41.4	4.0	27.8	28.6	84.4
NIAB-852-10	40	42.5	4.5	28.3	28.9	85.0
NIAB-852-20	68	38.9	3.9	28.1	29.2	86.0
NIAB-852-22	62	44.0	4.2	27.0	29.0	84.1
NIAB-852-25	41	43.0	3.9	27.5	29.0	80.0
NIAB-852-27	45	38.8	4.1	26.0	28.7	84.4

Discussion

The results of NIAB-852 have showed that, pollen irradiation of suitable parent before cross pollination is an appropriate technique to create genetic variability in cotton. The changes recorded in the selected mutant line are likely due to variation caused by gamma irradiation in the genetically controlled characters. These variations are may be due genetic modifications in the chromosomes i.e., aberrations, deletions, insertions etc or even changes in the sequence of some genes. Such types of genetic variations are earlier reported by different researchers. Muthusamy & Jayabalan (2000) recorded various changes in leaf shape in cotton. Whereas, Muthusamy *et al*. (2005) selected high yielding mutants in cotton. Twin boll and boll abnormalities and several other morphological variations were observed in cotton mutant lines (Muthusamy *et al.,* 2004; Muthusamy & Jayabalan, 2001).

The pollen irradiation approach seems good to create genetic variability. Male pollen is a germ cell and after irradiation followed by fertilization; half of the zygotic genome receives the irradiation. Therefore, occurrences of major changes in genetic material are minimized. Whereas in seed irradiation because the whole genome is affected by irradiation, therefore a large M_2 population (12,000 plants) are required, to select useful mutants (Iqbal *et al*., 1994). Whereas in the present study of pollen irradiation a small M_2 population (1000 plants) was required and higher frequency of mutants was recorded. Similar results are earlier reported by (Jalil & Yamaguchi, 1965; Vig, 1973). Pollen irradiation/treatment method is easier to apply than that seed irradiation. Male parent pollen irradiation before cross-pollination/hybridization created useful induced mutations in cotton. Such types of finding are earlier reported by (Pate & Duncan, 1963; Krishnaswami and Kothandaraman, 1976) and identified suitable mutants. Work on development and evaluation of cotton mutants developed through irradiation method is also reported by different researchers (Aslam & Stelly, 1994; Aslam, 2000; Aslam, 2002; Aslam &Elahi, 2000; Aslam *et al*., 2009, Haidar and Aslam, 2016). From the present results it is observed that useful genetic variability is created by the treatments of cotton pollen with gamma rays.

In the present experiment, 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, Cronn *et al*., 1999), which ultimately increases the growth and number of cells and the whole plant.

Due to irradiation effects, NIAB-852 exhibited 16.4% to 26.8% higher yield than standard CIM-496 in NCVT and PCCT. It also performed better than standard at national level as well. NIAB-852 has good boll bearing, rapid boll formation at later stages and better tolerance to cotton leaf curl virus (CLCuV) disease and insect's pests due to early maturity. It has better leaf foliage which is suitable for high density planting. 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 as well as international requirement of cotton lint and its products.

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