NIAB-777: AN EARLY MATURING, HIGH YIELDING AND BETTER QUALITY COTTON MUTANT DEVELOPED THROUGH POLLEN IRRADIATION TECHNIQUE - SUITABLE FOR HIGH DENSITY PLANTING

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ABSTRACT

This paper describes development of mutant NIAB-777 with improved yield and fibre characters through pollen irradiation method. A cross between NIAB-78 and REBA-288 using gamma irradiated pollen at the rate of 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 was achieved. Of these, M-7/02, later named as cv. NIAB-777 was finally selected. It produced 23.4% & 18.5% higher seed cotton yield from standards at local trials. It produced higher seed cotton yield in regional adaptability trials (17.4%), provincial coordinated cotton trials (16.6 %) and in national coordinated varietal trials (8.8 %) compared to standard cv. CIM-496. It has desirable fibre quality traits i.e. Ginning Out Turn (GOT) of 37.6%, fibre length of 28.81 mm, fibre fineness of 4.67 µg/inch, uniformity index of 85.0%, fibre maturity 81.2% and fibre strength 91.12 thousand pounds per square inch (TPPSI). NIAB-777 is suitable for high density planting. It has better tolerance to cotton leaf curl virus (CLCuV) disease and insects pests. It is concluded that 10 Gray gamma irradiation in cotton has effectively stimulate/increase the agronomical characters and tolerance to disease.

Key words: mutant, yield, fibre quality, cotton, pollen irradiation, NIAB-777.

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 a source of fibre, cattle feed, edible oil and almost all parts of cotton plants are used extensively in several industries but cotton is mainly cultivated for its fibre and seed oil (Sial *et al*., 2014). In Pakistan, cotton production is important both to earn foreign exchange earnings and for textile industry. The cotton production showed a remarkable increase during last 60 years and the textile mills have been increased from 2 to over 500. It is estimated that our textile industry would require 20 million bales of lint by 2020 (Haidar *et al*., 2007).

Cotton is cultivated over an area of 3125, 000 ha with an annual production of 12.8 million bales (Anonymous, 2013-14). A large of people in Pakistan is linked with cotton cultivation, ginning, oil industries, trade and spinning processes. Cotton producers in Pakistan are currently facing problem of rising production costs and static return.

Cotton belongs to genus *Gossypium* which makes around 50 species including four species that are known as cultivated (Cronn *et al*. 1999). The tetraplod specie *Gossypium hirsutum* L. is covering most of the cultivated area in world including Pakistan. It is cultivated on approximately 98% of total cultivated area under cotton crop in Pakistan. Our main focus of research in the present scenario is on *Gosspium hirsutum* which occupied mostly cultivated area.

Lot of efforts have been made by cotton researchers to develop cotton varieties having high yield potential, desirable fibre quality and tolerance/resistance to insect's pests and diseases through conventional breeding approaches. But still there is a long way to go. There are limitations of availability of sufficient genetic variability in the native germplasm (Haidar *et al*. 2012). There is an increased interest in the quantitative and qualitative improvement of cotton cultivars. The success of all conventional breeding approaches is highly correlated with the genetic variability present within the existing germplasm. 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 with improved characters of soybean (Hofmann *et al.* 2004), cotton (Muthusamy and Jayabalan, 2007), cassava (Joseph *et al.* 2004), *Chrysanthemum* (Datta *et al.* 2004), potato (Li *et al.* 2005) and groundnut

(Muthusamy *et al.* 2007) have been developed and released in the world through this technique (Ahloowaila *et al.* 2004).

The approaches like; the exposure of seed to ionizing radiations (Carnelius, 1973; Micke *et al*. 1987; Iqbal *et al*. 1991, 1994) and the treatment of pollen with low doses of gamma rays before cross-pollination resulted in creating genetic variability in cotton (Muthusamy and Jayabalan, 2011). They developed cotton mutants through treatment of gamma rays and EMS on immature ovules and investigated the effects of mutagenic treatments and have observed variations during the subsequent generations. These mutant lines showed significant variation from control lines. Moreover, lower dose application of mutagenic treatments effectively stimulate the agronomical characters like, early flowering, plant height, number of bolls, yield of seed cotton, ginning %age, seed index, harvest index and fibre characters (Haidar *et al*., 2016).

Generally in eukaryotic cells radiation treatments enhance crossing near the entromere region. Moreover, radiations as well as several chemicals are reported to increase somatic recombinations (Vig, 1973). Many plant breeding programmes have shown the feasibility of radiation plus selection as a direct method of varietal improvement. Irradiation of male parent pollen before cross- pollinations resulted in the induction of mutations in cotton (Haidar and Aslam, 2016). The studies carried out so for have shown that treatment of pollen with low doses of gamma rays (5 Gy to 20 Gy) before cross-pollinations are suitable to induce useful genetic variability in cotton (Yue and Zou, 2012).

The main objective of the study was to create genetic variability through crosses with irradiated male parent pollen and isolation and field evaluation of mutant line for better agronomic characters.

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 seventy plants of each genotype were developed. The plant material was obtained from the gene pool established at NIAB. The local cv. 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 is bushy type with average quality parameters but better tolerance/resistance to cotton leaf curl virus disease. At maturity flower buds of female parent were emasculated before anthesis during evening hours and covered with paper bags. Flower buds of male parent were also covered to protect any mixing. The cross was made by utilizing NIAB-78 (local cotton variety) as female parent with REBA-288 **(**exotic line) as male parent.

Radiation Treatment: Male parent pollen was collected from covered flowers after anthesis and irradiated with gamma rays 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 the seeds were obtained and designated as M_o seeds**.**

Evaluation of mutated generations: M_1 population was developed from M_o seed along with both parents as control at experimental fields of NIAB, Faisalabad. The seeds were planted at a spacing of 30 × 75cm plant to plant and row to row distance respectively. The 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 consisted 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 three replications by using Randomized Complete Block Design (RCBD). These progenies were studied in M_3 generation for their breeding behavior and economic traits and progeny was selected. Plant progeny rows were also studied in M_4 generation to confirm selection of desirable traits. Finally the progeny M-7/02 was selected from M_6 and bulked and named as NIAB-777. All these generations were raised and evaluated at Nuclear Institute for Agriculture and Biology (NIAB) where the soil type is clay loam having pH=7.2-7.5, EC value of 0.8-1.5 dS.m^{-1}and NPK used (60:23:23 kg/acre). Agronomic practices (hoeing, removal of weeds both by manually and use of weedicides, irrigations, application of fertilizers etc), were carried out to have uniform stand of crop and normal growth. Different plant protection measures by spray of insecticides/pesticides were carried out throughout the crop growing to control or minimize the sucking (thrips, jassid, whitefly, aphid) and bollworm (heliothious, spotted, pink and army bollworms) insect pests.

Evaluation in adaptability trials: Various trials (local yield trials, zonal yield trials i.e. NCVT, PCCT, 1.25 acre PSC farm etc.) were conducted at public sector experimental institutes etc. The objective was to analyze yield potential, fibre quality traits and wider adaptability in different climatic zones of Pakistan. Various characters to access the earliness of the selected mutant compared to standards were also recorded at NIAB.

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 inoculated with CLCuV-B through grafting by following the bottle shoot grafting method as described by Akhtar *et al*. (2002, 2010). 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 characters of the selected mutant lines were analyzed using High Volume Instrument (HVI) as well as manually operated instruments at NIAB, Faislabad. As per requirement of mandatory evaluation by Punjab Seed Council (PSC), fibre quality of NIAB-777 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 subjected to analysis of variance (ANOVA) using the methodology of 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

In this study, a local cotton variety was crossed with an exotic line by using gamma irradiated pollen to induce useful variation leading to development of mutant NIAB-777. Its developmental history is given in Table 1. The mutant NIAB-777 (Fig 1A) showed distinguishing features like earliness, medium height, more number of bolls, better opening and yield potential compared to parents NIAB-78 (Fig 1B) and REBA-288 (Fig 1C).

Influence of mutagenic treatments on selected plants: Significant differences were observed among the control and the plants developed from the irradiated pollen. There was maximum variation in M_2 generation and plants possessing desirable traits were selected. Succeeding M_3-M_6 generations were evaluated. The comparison of selected mutant from parents (control) is given in Table 2. The plants developed from the treated pollen showed different developmental features during seedling as well as normal plants development 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 differenced was recorded from seedling stage to maturity in M_2, M_3 and M_4 generations. Important morphological as well as yield characters along with fibre traits like ginning out turn percentage, staple length, fibre fineness, fibre strength, uniformity ratio percentage were selected to record and analyze the effect of irradiation treatments in comparison with the control (parents etc). The flowering periods of mutants were decreased as compared to the control and showed increase in yield. From the evaluated mutants, M-7/2 showed earliness and better seed cotton yield as compared to control. Similar results were earlier recorded by Swami and Swami, 1986. The plant height in selected mutants was 135-160 cm which was comparatively higher than untreated plants having a range of 125-150 cm. 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. The mutant line NIAB-777 produced 23.4 % & 18.5% higher seed cotton yield compared to standard cv. CIM-499 and cv. CIM-496 respectively at local trials at NIAB (Table 3). In regional adaptability trials in cotton growing regions, it produced 21.7% and 13.1% higher yield compared to cv. CIM-496 during two years testing (Table 4).

In provincial coordinated cotton trials (PCCT), NIAB-777 produced 11.0 % and 22.1% higher yield compared to cv. CIM-496 respectively during two years testing. Whereas in National Coordinated Varietal Trials (NCVT), NIAB-777 produced 9.4% and 8.7% higher yield at Punjab province locations (Table 4).

On an average of all mandatory trials, NIAB-777 produced seed cotton yield of 3470 $kg.ha^{-1}$ compared to standard variety cv. CIM-496 (2950 $kg.ha^{-1}$) producing 17.6 % higher seed cotton yield compared to standard. The data for seed cotton yield in various adaptability trials (PCCT, NCVT) showed significant differences through LSD test (Table 5). From these results it is observed that compared to standards and parents, NIAB-777 not only produced higher number of bolls but also produced significantly higher yield. Similar results were earlier reported in cotton by Nepolean, (1999).

***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-777 in comparison with cv. CIM-496. The results showed that the sympodia/plant of NIAB-777 were higher compared to cv. CIM-496 which confirmed higher number of bolls/plant. Moreover, the number of days taken towards maturity of NIAB-777are also less compared to cv. CIM-496. The same has been confirmed by higher percentage of seed cotton in 1st pick (81.6 %) compared to cv. CIM-496 (80%). During 2nd year studies it also produced more number of sympodia/plant and higher number of fruiting positions/boll retention which confirmed more number of bolls/plant compared to cv. CIM-496. It matures earlier compared to standard cv. CIM-496 (Table 6). Earlier Swami and Swami, 1986, found early flowering mutants in cotton by mutagenic treatment.

***Pathological Studies*:** Screening against cotton leaf curl virus (CLCuV) disease for the selected mutants was continued after selection. The finally selected mutant NIAB-777 was recorded resistant to CLCuV disease (old strain) like cv. CIM-499 and cv. CIM-496. The response against presently prevailing strain i.e. cotton leaf curl virus-Burewala (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. The results showed that NIAB-777 had better tolerance to CLCuV-B compared to cv. CIM-496 under natural field conditions with almost 100% disease intensity on susceptible lines. NIAB-777 had lower CLCuV-B disease incidence (19.4% to 21.9%) as compared to standard cv. CIM-496 (30.8 to 41.3%) during 1st year testing. During second year testing, NIAB-777 had also lower CLCuV-B disease incidence (18.5% to 25.6%) compared to standard cv. CIM-496 (55.8 to 79.2%) as given in (Table 7).

The response of NIAB-777 to CLCuV-B was also studied under natural disease inoculation. The observations showed that all the tested varieties showed varying disease intensity i.e. 16.8 to 68.5%. NIAB-777 with disease index of 32.2% showed better tolerance to CLCuV-B compared to cv. CIM-496 having 56.5% disease index (Table 8).

***Entomological Studies*:** Studies on NIAB-777 regarding its response to sucking pests and damage by bollworms were conducted under optimized spray condition at NIAB Faisalabad. It showed less population of sucking i.e. jassid (0.45/leaf), whitefly (2.05/leaf) and bollworms (11.7%) compared to standard cv. CIM-496 having higher population of sucking i.e. jassid (0.55/leaf), white fly (2.10/leaf) and bollworms (12.4%). During second year study, NIAB-777 also showed less population of sucking i.e. jassid (0.28/leaf), whitefly (4.78/leaf) and bollworms (12.14%) as compared to cv. CIM-496 having higher population of sucking i.e. jassid (0.31/leaf), white fly (4.93/leaf) and bollworms of 12.57% (Table 9).

***Fibre Quality Analysis*:** The results of fiber testing studies revealed that fibre quality traits of NIAB-777 are either better or comparable to standard cotton varieties (cv. CIM-499 & cv. CIM-496). Fibre testing at NIAB reflected that it has fibre length ranged from 28.5 to 29.0 mm, fibre fineness 4.2 to 4.6 µg/inch, fibre maturity 84.0 to 86.0% and fibre strength 92.5 to 96.0 TPPSI (Table 10).

Table 1. Developmental History of NIAB-777.

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 (M-7/02)	NIAB-777, bulked
M_7	studied in strain test
M_8	Preliminary Yield Trial
-	Advanced Yield Trial, PCCT,
-	NCVT, PCCT, 1.25 acre PSC trial
-	NCVT & 1.25 acre PSC trial, spot examination
Recommended by Expert subcommittee (ESC) & Approval by Punjab Seed Council (PSC)	
Provision of BNS to farmers and maintenance at NIAB	

Fibre quality of NIAB-777 was also 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. The results showed that on an average, NIAB 777 has fibre quality parameters i.e. GOT (37.56%), Fineness (4.67 µg/inch), fibre length (28.81mm), fibre strength (91.12 TPPSI, 31.6 G/Tex), uniformity index (length uniformity ratio) 85.0% and fibre maturity (81.2 %). All these fibre qualities are either better or at par with commercial standard cv. CIM-496 having fineness (4.77µg/inch), fibre length (28.91mm), fibre strength (87.4 TPPSI), uniformity index (length uniformity ratio) 84.32% and fibre maturity of 82.1 % It has better fibre quality parameters as compared to both parents (Table 11). Ginning out turn percentage, staple length, fineness, strength etc has been improved through irradiation treatment as compared to control parents. These observations were in confirmatory with earlier reports (Aslam *et al.,* 2009). Significant differences between individual tetraploid lines in terms of fibre contents were observed by Smith *et al.* (2004).

NIAB-777 was evaluated for high density planting i.e. at 9 inches, 12 inches, and 18 inches plant to plant spacing. NIAB-777 produced the highest yield at 9 inches spacing followed by NIAB-846 (Table 12). The best sowing time for NIAB-777 is recorded 1st May to 7th June.

It is maintained regularly at NIAB and Breeder Nucleus Seed (BNS) has been provided to farmers/seed producing agencies since its approval as commercial variety (Table 13).

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-777	145	0-3	75	3.5	220	37.56	28.81	4.67	91.12	31.60	85.0	81.2

Table 3. Yield performance of NIAB-777 in local NIAB trials.

Name of trial	Place	Yield (kg.ha^{-1})		% increase over check
		NIAB-777	CV. CIM-496	
Micro/Macro Varietal trials	NIAB, Faisalabad	4505	3481*	**1st year**
		5205	4385	**2nd Year**
Average		**4855**	**3933**	**29.4**
Advanced yield trials	NIAB, Faisalabad	5940	4946	**1st year**
		4069	3504	**2nd Year**
Average		**5004**	**4225**	**18.5**

Table 4. Yield performance of NIAB-777 in regional adaptability and national trials.

Name of trial	Place	Yield (kg.ha^{-1})		% increase over check
		NIAB-777	CV. CIM-496	
Regional Adaptability trials in Punjab-1st Year testing	i) CCRI, Multan	2808	2227	
	ii) RARI, Bahwalpur	1755	1683	
	iii) CRS, Sahiwal	910	589	
	Average	**1825**	**1500**	**21.7**
Regional Adaptability trials in Punjab-2nd Year testing	i) CRS, Sahiwal	2728	2834	
	ii) CCRI, Multan	2242	1453	
	iii) RARI, Bahwalpur	2517	2286	
	iv) PSC, Khanewal	3313	3377	
	v) CRS,Vehari	1443	892	
	Average	**2454**	**2168**	**13.1**
Average % increase in regional trials				**17.4**
PCCT (1st year)	Punjab	2501	2253	**11.0**
PCCT (2nd year)	Punjab	2761	2261	**22.1**
	Average	**2631**	**2257**	**16.6%**
NCVT (1st year)	Punjab + D. I. Khan	2479	2265	**9.4**
NCVT (2nd year)	Punjab + D.I. Khan	2962	2737	**8.7**
	Average	**2721**	**2501**	**8.8%**
Average seed cotton yield of NIAB-777 and CV. CIM-496		**3354**	**2891**	**16.0%**
Overall average yield of NIAB-777 and CV. CIM-499+ CV. CIM-496		**3470**	**2950**	**17.9%**

* Standards CV. CIM-499, CV. CIM-496
% increase in yield of NIAB-777 over CV. CIM-499 = 29.4%
% increase in yield of NIAB-777 over CV. CIM-496 = 16.0%
% increase in yield of NIAB-777 over CV. CIM-499 & CV. CIM-496 = 17.6%

Table 5. Average yield performance and test of significance of different candidate's varieties and standard varieties in PCCT and NCVT.

S/N	Variety	SCY ($kg.ha^{-1}$) 1st Year PCCT	Variety	SCY ($kg.ha^{-1}$) 2nd year PCCT	Variety	SCY ($kg.ha^{-1}$) 1st Year NCVT	Variety	SCY ($kg.ha^{-1}$) 2nd Year NCVT
1	VH-255	3054 A	FH-942	3007 BCDEF	CIM-554	2475 DEF	GH-102	2341 DE
2	FH-942	2711 AB	RH-620	2739 EFGHI	CRSM-38	2381 CDE	PB-900	2719 BCD
3	NIAB-846	2650 BC	VH-255*	2995 CDEF	TH-198/194	1864 HI	SLH-317	2695 ABC
4	MG-3	2601 BCD	CRSM-2007	3033 ABCDE	CIM-541	1704 I	NIAB-852	2818 BCD
5	FH-113	2569 BCDE	MG-6	2986 CDEF	RH-610	2476 DEF	CRIS-129	3007 ABC
6	CRSM-70	2542 BCDE	CIM-557	2553 HIJK	BH-167	2345 DEF	CRSM-38	2614 BCD
7	NIBGE115	2537 BCDE	GS-1	2305 JKL	**NIAB-777**	**2479** DEF	FH-942	2627 BCD
8	**NIAB-777**	2501 BCDE	VH-277	2270 KL	ASR-1	2278 EFG	CIM-554	2804 ABC
9	NIAB-852	2358 BCDEF	NIAB-852	2996 CDEF	CRIS-129	2940 A	**NIAB-777**	2962 ABC
10	SLH-284	2339 BCDEF	CRSM-38	2534 HIJK	CRSM-70	2478 EFG	TH-06/2	2116 E
11	CIM-554	2325 CDEF	VH-207	2807 DEFGH	GH-102	2325 EFG	NN-3	2764 BCD
12	BH-168	2302 CDEF	SLH-317	2608 HIJ	SLH-284	2799 ABC	NIA-78	2077 E
13	GS-1	2273 DEF	**CV.CIM-496**	**2261** KL	TH-86/02	2052 GHI	BH-172	3107 A
14	RH-610	2264 DEF	GS-14	2558 HIJK	GS-1	2465 DEF	CRSM2007	2590CD
15	CRSM-38	2257 DEF	CIM-554	2551 HIJK	NIAB-846	2648 BCD	FH-941	3074 AB
16	**CV.CIM-496**	2253 DEF	PB-900	2202 L	CRIS-342	2916 AB	GS-1	2418 DE
17	FH-941	2251 DEF	**NIAB-777**	2761 EFGHI	NIBGE-115	2814 AB	CIM-557	2857 ABC
18	BH-167	2248 DEF	SITARA-008*	2443 IJKL	**CV.CIM-496**	**2265** FGH	VH-278	2290 E
19	RH-541	2198 EF	A-One*	3247 ABC			GS-14	2691 BCD
20	ASR-1	2108 F	FH-941	3226 AB			**CV.CIM-496**	**2737** CD
21	VH-260	2025 FG	BH-172	2691 FGHI				
22	CIM-541	1703 GH	FH-2015	2973 CDEFG				
23	MG-2	1665 GH	NIAB-2008	**2663** GHI				
24	MG-1	1537 H	FH-113*	3108 ABCD				
25			NN-3	2757 EFGHI				
26			Alseemi-hybrid	3335 A				
CV %		19.78		13.87		8.51		17.41

Table 6. Earliness studies/Morphological traits of NIAB-777 recorded at NIAB.

Characteristics	NIAB-777		CV. CIM-496	
	1st Year	2nd year	1st Year	2nd year
Plant height (cm)	135-148	137-154	147-158	140-160
No. of sympodia/plant	28-35	27-32	22-28	20-25
First boll retention at node number	6-7	6-7	7-8	7-8
Total number of fruiting positions	258	260	208	204
Total number of shedding points	150	154	132	126
Total number of boll retention	108	106	76	78
Percentage of shedding points	58.1	59.2	63.4	61.7
Number of days taken to maturity	136-151	136-150	140-155	138-152
Seed cotton yield (1st pick %age)	81.6	80.0	80.0	78.0

Table 7. Response of NIAB-777 to CLCuV-B disease at NIAB.

Variety	Trial-1			Variety	Trial-2		
	Total plants	Affected plants	% age		Total plants	Affected plants	%age
1ST year Testing							
NIAB-777	292	64	21.9	NIAB-777	144	28	19.4
CV. CIM-496	174	72	41.3	CV. CIM-496	162	50	30.8
Mut. 725-4 (spreader)	69	69	100	Mut. 725-4(spreader)	66	66	100
2ND Year Testing							
NIAB-777	285	53	18.5	NIAB-777	2535	651	25.6
CV. CIM-496	267	149	55.8	CV. CIM-496	1250	990	79.2
Mut. 491-2(spreader)	45	45	100	Mut. 491-2(spreader)	120	120	100

Table 8. Field response of different cotton strains against CLCV-B under normal plant protection conditions in NCVT at NIAB Faisalabad.

Varieties	% Disease Index	Varieties	% Disease Index
FH-942	27.89	NN-3	18.56
CRSM-38	24.21	**CV. CIM-496**	**46.53**
CIM-557	25.68	BH-172	21.88
CRIS-129	28.49	PB-900	29.71
GH-102	51.97	GS-14	43.10
NIA-78	45.05	**NIAB-777**	**32.19**
NIAB-852	23.90	SLH-317	29.95
TH-06/2	59.17	GS-1	28.73
CRSM-2007	16.79	VH-278	68.50
CIM-554	32.29	FH-941	57.74

Table 9. Population of sucking insect pests, bollworms infestation of candidate varieties in NCVT.

Varieties	1st year study					Varieties	2nd year study				
	Sucking insects/leaf		Bollworms infestation (%)		Mean damage (% age Sq +Bolls)		Sucking insects/leaf		Bollworms infestation (%)		Mean damage (% age Sq +Bolls)
	Whitefly	Jassid	Squares	Bolls			Whitefly	Jassid	Squares	Bolls	
NIAB-777	2.05	0.45	16.23	7.20	11.72	NIAB-777	4.78	0.28	12.87	11.40	12.14
CV. CIM-496	2.10	0.55	14.76	10.04	12.40	CV. CIM-496	4.93	0.31	14.90	10.23	12.57

Table 10. Fibre traits of NIAB-777 tested at NIAB compared to standards

Year	NIAB-777					CV. CIM-499				
	GOT (%)	Length (mm)	Fineness (µg/inch)	Maturity (%)	Strength (TPPSI)	GOT (%)	Length (mm)	Fineness (µg/inch)	Maturity (%)	Strength (TPPSI)
1st year	38.5	29.0	4.4	85.0	95.0	38.5	28.5	4.6	82.7	94.0
2nd year	39.0	28.5	4.2	84.0	92.5	39.0	28.3	4.5	82.9	93.0
Average	**38.8**	**28.8**	**4.3**	**84.5**	**93.8**	**38.8**	**28.4**	**4.55**	**82.8**	**93.5**
	NIAB-777					**CV. CIM-496**				
1st year	38.4	29.0	4.2	85.0	96.0	41.5	27.9	4.7	83.0	87.0
2nd year	40.1	29.0	4.6	85.0	94.5	40.5	28.7	4.6	84.0	85.0
3rd year	39.0	29.1	4.5	86.0	96.0	40.0	28.2	4.7	82.0	87.0
Average	**39.1**	**29.0**	**4.4**	**85.3**	**95.5**	**40.6**	**28.3**	**4.7**	**83.0**	**86.3**

Table 11. Fibre traits of NIAB-777 tested during spot examination.

Lab./ Parameters	GOT	Staple Length	Fineness	Strength		U.I	Maturity
	(%)	(mm)	µg/inch	TPPSI	G/tex	(%)	(%)
CRI, Faisalabad	37.56	28.2	4.5	94.0	-	-	81.2
CCRI, Multan	-	29.6	**4.8**	92.0	31.2	85.9	-
NIBGE, Faisalabad	-	29.27	4.77	-	32.0		-
APTMA, Lahore	-	28.18	3.70	87.36	-	84.10	-
NIAB-777 (Average)	**37.56**	**28.81**	**4.67**	**91.12**	**31.60**	**85.0**	**81.2**
CV. CIM-496	43.29	28.91	4.77	82.80	29.45	84.32	82.1
CV. MNH-786	38.06	27.57	4.73	95.65	33.00	84.52	82.6

Table 12. Performance of NIAB-777 under high density at NIAB.

Plant to plant spacing (cm)	Seed cotton yield ($kg.ha^{-1}$)		
	NIAB-777	NIAB-824	CV. NIAB-846
9	5715.9	5334.9	5467.3
12	5310.2	4959.8	5156.5
18	4800.0	4750.0	5050.5

Table 13. Maintenance and provision of Breeder Nucleus seed (BNS) of NIAB-777.

Variety	BNS (Kg) provided to cotton growers/seed distribution agencies					
	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16
NIAB-777	728	847	280	62	29.0	10
	Fiber quality characters evaluated during 2015					
Progeny Bulks of NIAB-777	No. of bolls/ plant	GOT (%)	Fibre Fineness (µg/inch)	Fibre Strength (g/Tex)	Fibre length (mm)	Uniformity ratio (%)
NIAB-777-2	52	38.4	4.5	27.7	28.0	86.4
NIAB-777-8	58	40.0	4.4	31.9	28.3	84.7
NIAB-777-9	68	40.3	4.1	28.6	28.0	85.5
NIAB-777-17	56	38.0	4.8	29.5	29.5	86.6
NIAB-777-23	66	43.1	4.7	26.0	28.3	83.5

Figure 1. Mutant NIAB-777(A) in comparison with parents NIAB-78 (B) and REBA-288 (C) with plant type, branching and fruiting pattern.

DISCUSSION

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. The changes recorded in the selected mutant line may be due to genetic variation caused by gamma irradiation. Such types of genetic variations are earlier reported by different researchers. Muthusamy and Jayabalan (2000) recorded number of variations 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 and Jayabalan, 2001).

The pollen irradiation approach seems good to create genetic variability. 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. In case of seed irradiation since the whole genome is affected, hence a large M_2 population (approximately 12,000 individual plants) are required, to select desirable mutants (Iqbal, *et al*. 1994). Whereas in the present study of pollen irradiation a very small M_2 population (even less compared to 1000 plants) was required and higher rate of mutations/recombinations was achieved. Similar results are earlier reported by (Jalil and Yamaguchi, 1965; Vig, 1973). Pollen/gamete treatment method is found easier to apply compared to that of zygote/seed treatment. Irradiation of male parent pollen before cross-pollinations resulted in the induction of mutations in cotton. Such types of finding are earlier reported by (Pate and 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 and Stelly, 1994; Aslam *et al*., 1994; Aslam, 2000; Aslam, 2002; Aslam and Elahi, 2000). From the present results it is observed that the treatments of pollen with low doses of gamma rays are suitable to induce useful genetic variability in cotton

In the present experiment, lower dose of gamma irradiation showed enhancing effects on growth of vegetative parts of plants along with floral and yield characters. It may be due to increased activity of enzymes involved in biosynthesis of hormone in the cell (Vagera et al, 1976), which increases the growth of cells and ultimately the whole plant.

Due to irradiation effects, NIAB-777 exhibited 8.7% to 23.4% higher yield compared to standard cv. CIM-496 in NCVT and PCCT. NIAB-777 being moderately hairy with medium sized erect plant type with short to medium short sympodia and better leaf foliage 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.

Conclusion: NIAB-777 has better plant type, desirable leaf foliage, high yield potential, earliness, better tolerance to CLCuV disease along with good fibre characteristics and hence was recommended to farmers for cultivation. Its cultivation will be adding to the national exchequer through export of raw cotton and value added products and to meet the national and international demands.

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