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NIAB-846: HIGH YIELDING AND BETTER QUALITY COTTON MUTANT DEVELOPED THROUGH POLLEN IRRADIATION TECHNIQUE

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This paper describes about cotton mutant NIAB-846 developed through pollen irradiation technique with improved yield and fibre quality parameters. A cross was attempted between NIAB-78 (local variety) and REBA-288 (an exotic line) at NIAB. The pollen used for crossing was irradiated @10 Gy (Grey) of gamma rays before cross pollination. The objective of induced mutations through pollen irradiation was to develop new germplasm and select new cotton mutants having desirable traits. The M_2 population was raised using M_1 seeds and mutants with better yield, earliness, resistance/tolerance to diseases and insect pests were selected. The selected mutants were evaluated for higher yield and yield contributing traits in different generations (M_3-M_6). Significant variations compared to parents were observed in evaluated mutated generations. Among the selected mutants, M-46/02 later named as NIAB-846 was finally selected for field performance evaluation. It produced 32.2% higher seed cotton yield compared to standard CIM-499 and CIM-496 at NIAB local trials. In regional adaptability trials in different zones of Punjab, it produced 22.4% higher seed cotton yield than standards. Whereas, in provincial coordinated cotton trials (PCCT) and in national coordinated varietal trials (NCVT), it produced 7.0 % and 8.0 % higher seed cotton yield respectively compared to standards. At Punjab locations it produced higher seed cotton yield than standard and ranked 4th in yield over all the candidate lines. On an average of all trials, NIAB-846 produced 18.9 % higher seed cotton yield. The mutant NIAB-846 has desirable fibre quality traits i.e. ginning out turn (GOT) 38.49%, fibre length 29.80 mm, fibre fineness 4.67 µg/inch, uniformity index 85.5%, fibre maturity 83.0% and fibre strength 96.1 thousand pounds per square inch (TPPSI). NIAB-846 is high yielding, early maturing with desirable plant type. It has sympodial type with one to two fruit bearing monopodia, desirable foliage and medium stature. It has better tolerance to cotton leaf curl virus-burewala strain (CLCuV-B) disease and insect pests. It is maintained and breeder nucleus seed (BNS) provision to farmers is in progress. From these results it is concluded that low dose pollen irradiation technique in cotton is effectively used to enhance/increase the different yield and yield contributing traits, fibre quality and tolerance to diseases in cotton. This technique is also proved to be economical as compared to transformation/transgenic procedures for plant improvement.

Keywords: NIAB-846, mutant, yield, fibre quality, cotton, pollen irradiation.

INTRODUCTION

Pakistan is one of the most prominent cotton producing and consuming country of the world. Cotton production is not only important due to foreign exchange earning but also is more important for our textile industry as cheaper raw material. Almost all parts of cotton are used but it is mainly cultivated for its fibre and seed oil (Sial *et al*., 2014). However, presently Pakistan is importing cotton lint to meet the demand of its textile industry by spending huge amount of foreign exchange. In spite of the fact that over the past 60 years, the cotton production showed a remarkable increase but the textile mills have been increased from 2 to over 500. It is estimated that our textile industry would require 25 million bales of lint by 2020 (Haidar *et al*., 2007).

Considering the importance of cotton in the country's economy, it is cultivated on an area of 3.125 million hacters with an annual production of 12.8 million bales (Anonymous, 2014) which is less as per our requirement. Overall, the living of millions of people in Pakistan is 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).

Cotton falls in genus *Gossypium* has around 50 species including four species that are known as cultivated and have been domesticated for their fiber production. Among these two are diploid with ploidy level of $2n=2x=26$ and two are tetraploid having ploidy level of $2n=4x=52$ (Cronn *et al*., 1999). Tetraploid cotton specie (*Gossypium hirsutum* L) is covering most of the cultivated area in the world including Pakistan. Approximately 98% of total cultivated area under cotton crop in under tertaploid specie (*Gossypium hirsutum* L) in Pakistan. Our main focus of research in the present scenario is on this specie.

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). Selection of characters like, early flowering, different morphological traits and quality parameters play a very important role to improve yield in cotton. Irradiation of male parent pollen before cross-pollinations resulted in the induction of mutations in cotton like earliness, fibre quality and yield contributing traits (Haidar *et al*., 2016; Haidar and Aslam, 2016, Aslam *et al*., 2018). Similarly, treatment of pollen with low doses of gamma rays (5 Gy to 20 Gy) before cross-pollinations is suitable to induce useful genetic variability in different morphological traits in cotton (Yue and Zou, 2012).

To achieve desirable objectives through conventional breeding approaches is depended on the existing genetic variability present within the already available germplasm resources. If the genrmplasm/desired trait is not available within the existing resources and or linked with other undesirable traits, then the cross breeding may not be effective to achieve the set target. In such cases recombination of genes is to be sought out to achieve the desired objectives. To achieve such objectives is highly depended on adopted techniques. Raising of induced variants/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). Recombination of genes during cell division process plays an important role to induce useful genetic changes and especially for linked genes is brought about by crossing over. The approaches like; exposure of seed to ionizing radiations (Maluszynski *et al*., 1995; Iqbal *et al*., 1991, 1994; Micke *et al*., 1987; Carnelius, 1973) and the irradiation treatment of male pollen with low doses of gamma rays before cross-pollination resulted in the development of new genetic changes/variability in different crop species. In addition to radiations, several chemicals mutagens are also reported to increase recombinations in somatic cells (Vig, 1973). Number of mutants with improved characters of different plants have been developed and released in the world through this technique (Ahloowaila *et al.,* 2004). Such improvements were reported in different crops like groundnut (Muthusamy *et al.,* 2007), cotton (Muthusamy and Jayabalan, 2007), potato (Li *et al.,* 2005), cassava (Joseph *et al.,* 2004), soybean (Hofmann *et al.,* 2004) and *Chrysanthemum* (Datta *et al.,* 2004).

Muthusamy and Jayabalan (2011) developed cotton mutants through treatment of gamma rays and observed variations during the subsequent generations. The use of mutagenic treatments enhance the important variation and improvements in characters like, early flowering, plant height and other yield contributing and fibre characters. Due to the adoption of this technique, many plant research programs related to plant breeding have shown good results and the feasibility of radiation plus selection as a direct method of varietal improvement.

The objective of the present study was to create genetic variability through crosses with irradiated male parent pollen and selection of desirable mutants/recombinants from the segregating populations. Evaluation of selected mutant for yield potential, fibre quality, adaptability and stability in the cotton growing areas and finally recommend to farmers for its cultivation. This manuscript details the report of induction of mutations in cotton using low doses of gamma irradiation on germ cells and selection & evaluation of desirable cotton mutants.

MATERIALS AND METHODS

***Plant material and radiation treatment*:** Selfed seeds of locally approved variety NIAB-78 and an exotic line (REBA-288) were planted at cotton experimental area of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan and approximately two hundred plants of each parent genotype were developed. The local variety NIAB-78 has good agronomic traits, average quality but susceptible to CLCuV disease. Whereas the exotic line REBA-288 has bushy type plant, average quality parameters but better tolerance to CLCuV disease. At evening time flower buds of female parent were emasculated and covered with paper bags. Male parent flower buds were also covered to protect any mixing. The cross between NIAB-78 as female parent with REBA-288 as male parent was attempted by using irradiated male pollen. Male parent pollen was collected from covered flowers after anthesis and irradiated with gamma rays from ^{60}Co irradiation source at NIAB, Faisalabad. The irradiation was performed at room temperature. Emasculated flowers were pollinated with irradiated pollen and rebagged to prevent uncontrolled crossing. Bolls developed from the crossed flowers were picked; and seeds were separated and designated as M_0 seed**.**

***Field evaluation of mutated generations and adaptability trials*:** M_1 population was developed from M_0 seed alongwith both parents as control at experimental field of NIAB, Faisalabad. The seeds were planted at a spacing of 30 x 75cm P x P and R x R distance respectively. From M_1 generation seeds were collected from the matured bolls. Characteristics of plants were recorded in M_1 generation plants along with control/non-irradiated parent plants. The M_2 population was raised from M_1 seed which 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, collectively plants were selected according to applied dose and carried forward as one locule bulk basis. Whereas in succeeding generations (M_2, M_3, M_4) single plants were selected and forwarded as single plant progeny rows. Thirty-five selected mutants were planted in M_3 generation in three replications. These progenies were studied in M_3 generation for their breeding behavior and best progenies were selected. Plant progeny rows were also evaluated in M_4 generation to confirm its desirable traits. Finally, the progeny M-46/02 was selected from M_6, bulked and named as NIAB-846. All these generations were raised and evaluated at NIAB where the soil type is clay loam. Agronomic practices were carried out to have uniform stand of crop and normal growth. Different plant protection measures were carried out throughout the crop growing season to control or minimize the sucking and bollworm insect pests.

The selected mutant NIAB-846 was evaluated in various trials (local yield trials, zonal yield trials i.e. national coordinated varietal trials (NCVT), provincial coordinated cotton trials (PCCT), 1.25 acre PSC farm trial etc.) at public sector experimental institutes. The objective was to analyze yield potential, fibre quality traits and wider adaptability in different climatic zones of Pakistan. Earliness studies were also conducted at NIAB.

***Screening for cotton leaf curl virus disease (CLCuV) and insect pests*:** Screening against CLCuV disease was done through grafting and whitefly inoculation technique. For grafting studies, ten pots of each variety were sown in glass house. Plants were inoculated/infected with CLCuV through grafting by using the bottle shoot grafting technique as earlier used 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.

***Fiber character analysis*:** Fibre characters of the mutants were recorded by 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 parameters of NIAB-846 were analyzed in four standard laboratories i.e. Cotton Research Institute (CRI), Faisalabad; PCS Institute, Multan; PICR&T, PCCC Karachi 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 analyzed using HVI.

***Statistical Analysis*:** The experiments related to yield evaluation were planted in randomized complete 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 using analysis of variance (ANOVA) as earlier described by 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, local and exotic cotton varieties were crossed using gamma irradiated male pollen to induce useful variation leading to development of mutant NIAB-846. Its developmental history is given in Table 1. The mutant NIAB-846 (Fig 1A) showed distinguishing features 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.

Table 1. Developmental History of NIAB-846

Parentage/Pedigree/ Generation	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-46/02, bulked, NIAB-846
M_7	studied in strain test
M_8	Preliminary Yield Trial (PYT)
M_9	Advanced Yield Trial (AYT)
-	NCVT, PCCT, 1.25 acre PSC trial
-	NCVT, PCCT, 1.25 acre PSC trial, Spot examination
Recommended by Expert subcommittee (ESC), approval by Punjab Seed Council (PSC), dissemination of BNS seed to farmers	

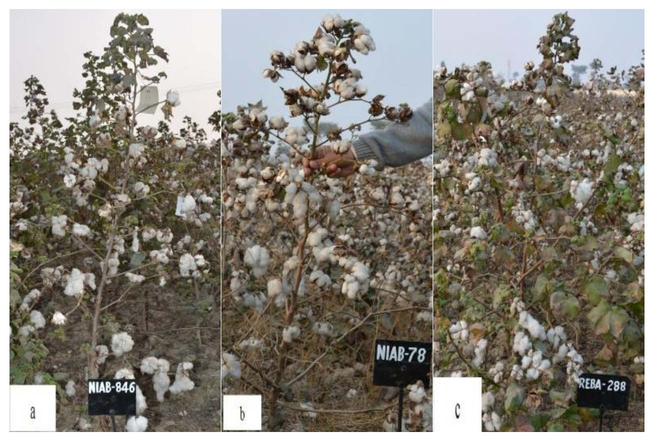

Figure 1. Mutant NIAB-846(A) in comparison with parents NIAB-78 (B) and REBA-288 (C) with plant type, branching and fruiting pattern.

***Influence of mutagenic treatments and field performance of mutant lines*:** Significant differences were recorded between the parent plants and the mutant developed from the irradiated pollen. There was maximum variation in M_2 generation and plants possessing desirable traits were selected. Succeeding generations M_3-M_6 were evaluated, and as a result M-46/02 with high yield potential was selected which was later named as NIAB-846. The comparison of selected mutant from parents (control) is given in Table 2. The plants developed from the irradiated pollen reflected different plant traits at different growth stages in the field conditions.

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-846	155	0-2	85	3.5	235	38.49	29.80	4.67	96.1	32.0	85.5	83.0

Table 3. Yield performance of NIAB-846 compared with standard varieties at NIAB

Name of trial	Place	Yield ($kg.ha^{-1}$)		% increase over check
		NIAB-846	CIM-496	
Micro/Macro Varietal trials	NIAB, Faisalabad	4775.5	3554.2	
		4303.4	3564.4	
		4515.6	3658.0	
	Average	4532.0	3592.0*	26.0%
Advanced yield trials	NIAB, Faisalabad	5159.4	3714.0	
		5590.5	4061.0	
	Average	5375.0	3888.0	38.3%
		Average % increase in local trials		32.2%

Morphological variations were observed from seedling to maturity stage in M_2, M_3 and M_4 generations. Some of the morphological as well as yield characters along with fibre traits like GOT (%age), fiber length, fineness, strength, uniformity ratio percentage were recorded and analyzed the effect of pollen irradiation treatments in comparison with control (parents etc). The flowering periods of pollen irradiated mutants were decreased than control but showed an increase in yield. From the evaluated mutants, M-46/02 showed earliness and better seed cotton yield as compared to control. The plant height in selected mutants was 148-163 cm which was comparatively higher than untreated plants having a range of 145-158 cm.

As per requirement of mandatory evaluation of finally selected mutant, it was evaluated in various provincial and national coordinated trials to check its yield potential and adaptability. In local trials it produced 26.0 % and 38.3% higher seed cotton yield than standards fixed at national levels i.e. CIM-499 CIM-496 respectively (Table 3). In regional trials it produced 16.3% and 28.4% higher seed cotton yield than CIM-496 during two years testing respectively. In provincial coordinated cotton trials (PCCT), it produced 17.6% higher yield during second year. In national coordinated cotton trials (NCVT), NIAB-846 ranked 4th in yield on Punjab basis and produced 4.9% and 11.1% higher yield than CIM-496 during two years of testing. On an average of all trials, it produced 18.9 % higher seed cotton yield compared to standard (Table 4). 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 than NIAB-846 not only produced higher number of bolls but also produced significantly higher yield in comparison with the standard varieties.

Earliness Studies**:** Studies were carried out on NIAB-846 for earliness as compared to standard CIM-496 and different morphological traits i.e. plant height, sympodia/plant, first boll retention at node number, total fruiting points and shedding points etc, were recorded. The results revealed that the traits (plant height, sympodia/plant) of NIAB-846 are higher than standard which confirmed more number of bolls/plant. Moreover, the results indicated that the numbers of days taken to maturity of NIAB-846 are also at par with standard. During 2nd year study, plant height and the sympodia/plant of NIAB-846 are slightly higher than standard and number of days taken to maturity are almost at par with standard. Moreover, in case of NIAB-846, the numbers of bolls/plant were higher than standard which was due to higher number of fruiting positions/boll retention than standard (Table 6).

Pathological and Entomological Studies**:** Evaluation of NIAB-846 against CLCuV disease was continued after selection. The results revealed that NIAB-846 was resistant to CLCuV disease (old strain) like standards CIM-499 and CIM-496. The response of NIAB-846 to latest strain of cotton leaf curl-Burewala strain (CLCuV-B) disease was also studied. The most susceptible spreader mutants/lines were also planted. The results showed that NIAB-846 had better tolerance to CLCuV-B than CIM-496 under natural field conditions with almost 100% disease intensity on susceptible lines. NIAB-846 had lower CLCuV-B disease incidence (5.3% to 11.4%) as compared to standard CIM-496 (20.0 to 47.9%). During 2nd year study, NIAB-846 had also lower CLCuV-B disease incidence (10.7% to 11.0%) as compared to standard having 17.8 to 41.4% (Table 7). The response of NIAB-846 to CLCuV-B was also studied in NCVT under natural disease inoculation of non-sprayed conditions.

Table 4. Yield performance of NIAB-846 compared with standard varieties in regional trials

Name of trial	Place	Yield (kg.ha^{-1})		% increase over check
		NIAB-846	CIM-496	
Regional Adaptability trials in Punjab-1st Year testing	i) CCS, Vehari	1665.3	1478.2	
	ii) CCRI, Multan	2732.5	2163.2	
	iii) RARI, Bahwalpur	2708.0	2468.0	
	Average	2369.0	2036.0	16.3%
Regional Adaptability trials in Punjab-2nd Year testing	i) CRS, Sahiwal	1039.5	589.1	
	ii) CCRI, Multan	2894.2	2227.1	
	iii) RARI, Bahwalpur	1845.0	1683.0	
	Average	1926.0	1500.0	28.4%
	Average % increase in regional trials			22.4%
PCCT (1st year)	Punjab	2351.0	2421.0	-3.0%
PCCT (2nd year)	Punjab	2650.0	2253.0	17.6%
	Average	2501.0	2337.0	7.0%
NCVT (1st year)	Pakistan (Punjab basis)	2730.0	2603.0	4.9%
NCVT (2nd year)	Pakistan	2404.0	2164.0	11.1%
	Average	2567.0	2384.0	8.0%
Average seed cotton yield of NIAB-846 and CIM-496		2829.2	2409.3	17.4%
Overall average yield of NIAB-846 and CIM-499+ CIM-496		3042.0	2557.1	18.9%

* Standard CIM-499, % increase in yield of NIAB-846 over CIM-499 = 26.1%, % increase in yield of NIAB-846 over CIM-496 = 17.4%, % increase in yield of NIAB-846 over CIM-499 & CIM-496 = 18.9 %

Table 5. Average yield performance and significance of different candidate and standard varieties in provincial and national trials

S #	Variety	SCY (kg/ha) in 1ST Year PCCT	Variety	SCY (kg/ha) in 2nd year PCCT	Variety	SCY (kg/ha)in 1styear NCVT	Variety	SCY (kg/ha) in 2nd year NCVT
1	FH-113	2672 A	VH-255	3054 A	NIBGE-115	2396 BCD	CIM-554	2201 DEF
2	MNH-786	2601 A	FH-942	2711 AB	MJ-6	2336 BCD	CRSM-38	1976 CDE
3	FH-941	2545 AB	NIAB-846	2650 BC	GH-99	2219 CD	TH-198/194	1818 HI
4	FH-942	2499 AB	MG-3	2601 BCD	CIM-541	2327 BCD	CIM-541	1782 I
5	NIAB-777	2478 AB	FH-113	2569 B-E	CIM-496	2511 A-D	RH-610	2218 DEF
6	CIM-496	2421 AB	CRSM-70	2542 B-E	TH-35/99	2617 ABC	BH-167	2148 DEF
7	RH-514	2382 AB	NIBGE115	2537 B-E	MNH-786	2721 AB	NIAB-777	2255 DEF
8	NIAB-846	2351 AB	NIAB-777	2501 B-E	CIM-538	2515 A-D	ASR-1	2067 EFG
9	FH-207	2322 AB	NIAB-852	2358 B-F	NIAB-824	2459 BCD	CRIS-129	2636 A
10	NIBGE-115	2302 AB	SLH-284	2339 B-F	FH-127	2190 CD	CRSM-70	2157 EFG
11	MNH-6070	2280 AB	CIM-554	2325 C-F	BH-167	2111 D	GH-102	2212 EFG
12	SLH-284	2260 ABC	BH-168	2302 C-F	CRIS-342	2730 AB	SLH-284	2472 ABC
13	NIAB-824	2237 ABC	GS-1	2273 DEF	NIAB-846	2381 BCD	TH-86/02	1956 GHI
14	VH-156	2191 ABC	RH-610	2264 DEF	ASR-1	2387 BCD	GS-1	2347 DEF
15	MNH-789	2164 ABC	CRSM-38	2257 DEF	TH-84/99	2162 D	NIAB-846	2404 BCD
16	MJ-6	2161 ABC	CIM-496	2253 DEF	CRIS-466	2306 BCD	CRIS-342	2594 AB
17	NIBGE-4	2151 ABC	FH-941	2251 DEF	FH-113	2907 A	NIBGE-115	2319 AB
18	VH-209	2088 ABC	BH-167	2248 DEF	NIBGE-4	2539 A-D	CIM-496	2164 FGH
19	VH-148	2072 ABC	RH-541	2198 EF	SLH-284	2527 A-D		
20	ASR-1	2013 ABC	ASR-1	2108 F	MNH-789	2205 CD		
21	BH-168	1983 BC	VH-260	2025 FG				
22	BH-167	1967 BC	CIM-541	1703 GH				
23	CIM-541	1967 BC	MG-2	1665 GH				
24	CIM-538	1668 C	MG-1	1537 H				
CV%	16.5			21.78		12.56		8.51

Table 6. Earliness studies/Morphological traits of NIAB-846 recorded at NIAB

Characteristics	NIAB-846		CIM-496	
	1st year	2nd year	1st year	2nd year
Date of sowing	01-06-2006	29-05-2007	01-06-2006	29-05-2007
Plant height (cm)	148-160	150-163	145-150	147-158
No. of sympodia/plant	25-30	25-30	22-26	22-28
First boll retention at node number	7	7	8	8
Total number of fruiting positions	230	250	192	208
Total number of shedding points	152	155	125	132
Total number of boll retention	78	95	67	76
Percentage of shedding points	66.0	64.5	65.1	63.4
Number of days taken to maturity	140-160	140-155	145-155	140-150
Seed cotton yield (1st pick %age)	72.8	76.8	77.5	80.0

Table 7. Response of NIAB-846 to CLCuV disease incidence at NIAB, Faisalabad

Variety	Trial-1			Variety	Trial-2		
	Total plants	Affected plants	% age		Total plants	Affected plants	Total plants
1st year study							
NIAB-846	306	35	11.4	NIAB-846	114	6	5.3
CIM-496	73	35	47.9	CIM-496	65	13	20.0
P50x443-10-2 (spreader)	113	113	100.0	P50x443-10-2 (spreader)	45	45	100.0
2nd year study							
NIAB-846	308	34	11.0	NIAB-846	992	106	10.7
CIM-496	58	24	41.4	CIM-496	410	73	17.8
P50x443-10-2 (spreader)	21	21	100	P50x443-10-2 (spreader)	20	20	100.0

Final observations showed that all the varieties were showing disease intensity from 18.26 to 76.3 %. NIAB-846 with disease intensity of 39.72% compared to standard (42.56%) showed better tolerance to CLCuV-B (Table 8).

Table 8. Field response of different cotton strains against CLCuV under normal plant protection conditions in national and provincial trials at NIAB

NCVT			
Varieties	% Disease Index	Varieties	% Disease Index
CRIS-129	22.77	CIM-541	60.82
GH-102	64.93	CRIS-342	37.00
TH-86/02	76.33	V13	42.53
V4	31.33	CRSM-70	43.95
CRSM-38	39.84	TH-198-94	74.37
CIM-554	57.65	SLH-284	65.56
NIAB-777	64.56	ASR-1	54.08
GS-1	47.77	NIBGE-115	18.26
NIAB-846	**39.79**	BH-167	67.45
CIM-496	**42.56**	RH-610	62.42

In studies regarding response to sucking pests and damage by bollworms were conducted under optimized spray condition at NIAB Faisalabad. NIAB-846 showed less population of sucking i.e. jassid (0.36/leaf), whitefly (2.06/leaf) and bollworms (12.52%) as compared to standard CIM-496 having population of sucking i.e. jassid (0.55/leaf), white fly (2.10/leaf) and bollworms 12.4% (Table 9).

***Fibre Quality Analysis*:** The results of fiber testing studies revealed that fibre quality traits of NIAB-846 are either better or comparable to standards (CIM-499 & CIM-496) tested at NIAB. Its fibre length ranged from 29.4 to 29.7 mm, fibre fineness 4.1 to 4.5 µg/inch, fibre maturity 82.3 to 84.2% and fibre strength 93.0 to 97.0 TPPSI (Table 10). Fibre quality of NIAB-846 was also analyzed by four standard laboratories (CRI, Faisalabad, CCRI, Multan, PCS Institute, Multan and PICR&T, PCCC Karachi) from the samples collected during spot examination of expert's sub-committee of PSC. The results showed that on an average, NIAB 846 scored fibre quality parameters i.e. GOT (38.49), Fineness (4.67 µg/inch), fibre length (29.80 mm), fibre strength (96.1 TPPSI, 32.0 G/Tex), uniformity index (length uniformity ratio) 85.5% and fibre maturity (83.0 %). All these fibre qualities are either better or at par with commercial standard CIM-496 having fineness (4.59µg/inch), fibre length (29.63mm), fibre strength (87.4 TPPSI), uniformity index (length uniformity ratio)

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

Varieties	Sucking insects/leaf		Bollworms infestation (%)		Mean damage
	Whitefly	Jassid	Squares	Bolls	(% age Sq +Bolls)
CRIS-129	1.97	0.48	15.08	9.02	12.05
GH-102	2.28	0.57	16.10	10.75	13.43
TH-86/02	2.08	0.46	13.52	6.74	10.13
V4	2.05	0.43	14.54	7.36	10.95
CRSM-38	1.98	0.44	13.95	6.05	10.00
CIM-554	2.19	0.57	16.32	7.47	11.90
NIAB-777	2.05	0.45	16.23	7.20	11.72
GS-1	2.06	0.47	15.30	6.11	10.71
NIAB-846	**2.06**	**0.36**	**15.98**	**9.05**	**12.52**
CIM-496	**2.10**	**0.55**	**14.76**	**10.04**	**12.40**
CIM-541	1.79	0.41	16.31	7.50	11.91
CRIS-342	2.12	0.57	12.24	6.16	9.20
V13	2.05	0.53	14.66	6.00	9.32
CRSM-70	2.22	0.41	14.61	9.34	12.00
TH-198-94	1.91	0.53	15.83	6.73	10.67
SLH-284	1.91	0.50	14.30	13.84	14.87
ASR-1	2.11	0.47	15.39	8.18	11.24
NIBGE-115	2.10	0.52	12.77	10.29	12.84
BH-167	2.00	0.40	14.45	7.12	9.95
RH-610	2.10	0.49		8.11	11.28

Table 10. Fibre traits of NIAB-846 tested at NIAB compared to standards

Year	NIAB-846					CIM-499*				
	GOT (%)	Length (mm)	Fineness (µg/inch)	Maturity (%)	Strength (TPPSI)	GOT (%)	Length (mm)	Fineness (µg/inch)	Maturity (%)	Strength (TPPSI)
2003-04*	38.0	29.4	4.1	82.3	94.0	38.5	28.0	4.5	81.5	93.0
2004-05*	38.2	29.7	4.0	83.5	93.5	38.3	28.3	4.5	82.7	92.0
2005-06*	38.4	29.6	4.1	84.0	93.0	38.2	28.3	4.5	82.9	92.0
Average	**38.2**	**29.6**	**4.1**	**83.0**	**93.5**	**38.3**	**28.2**	**4.5**	**82.4**	**92.3**
	NIAB-846					**CIM-496****				
2006-07**	38.9	29.7	4.1	84.2	95.0	38.0	27.5	4.6	81.0	94.0
2007-08**	38.9	29.6	4.5	82.8	97.0	39.4	28.7	4.6	82.0	95.5
Average	38.9	29.7	4.3	83.5	96.0	38.7	28.1	4.6	81.5	94.8

Table 11. Fibre traits of NIAB-846 tested during spot examination

Lab./ Parameters	GOT (%)	Staple Length (mm)	Fineness µg/inch	Strength		U.I (%)	Maturity (%)
				TPPSI	G/tex		
CRI, Faisalabad	38.49	29.5	4.6	97.8	-	-	81.5
CCRI, Multan	-	31.1	5.1	99.7	31.3	85.6	-
PCS Institute, Multan	-	28.8	5.19	-	32.7	85.4	-
PICR&T, PCCC Karachi	-	-	3.80	90.8	-	84.5	-
NIAB-846 (Average)	**38.49**	**29.80**	**4.67**	**96.10**	**32.0**	**85.5**	**83.0**
CIM-496 (St)	40.12	29.63	4.59	87.40	29.45	86.0	80.6
MNH-786 (St)	38.92	27.77	4.75	100.1	33.00	84.5	83.1

86.0% and fibre maturity of 80.6 % (Table 11). Ginning out turn percentage, staple length, fineness, strength was improved through irradiation treatment as compared to control parents.

***Planting Density Studies and Dissemination to Framers*:** NIAB-846 was evaluated for high density planting i.e. 6, 12 and 18 inches. NIAB-846 produced the highest yield at 18 inches (45cm) spacing followed by yield at 6 inches (15cm) at the same nitrogen level. However, control produced the

Table 12. Maintenance and provision of Breeder Nucleus seed (BNS) of NIAB-777 during 2009-2015

Variety	**BNS (Kg) provided to cotton growers/seed distribution agencies**							
	2009-10	**2010-11**	**2011-12**	**2012-13**	**2013-14**	**2014-15**	**2015-16**	**2016-17**
NIAB-846	563	469	530	170	25	9.0	6 .0	7.0

	Fiber quality characters evaluated during 2015-16					
Progeny Bulks of NIAB-846	**No. of bolls/ plant**	**GOT (%)**	**Fibre Fineness (µg/inch)**	**Fiber Strength (g/Tex)**	**Fiber length (mm)**	**Uniformity ratio (%)**
NIAB-846-3	37	37.5	4.3	33.6	28.5	84.2
NIAB-846-6	58	37.0	4.9	35.9	28.1	85.9
NIAB-846-9	68	39.4	4.6	34.7	28.4	84.5
NIAB-846-10	48	41.6	4.1	37.2	29.0	85.1
NIAB-846-11	52	38.0	5.0	34.6	28.5	85.9
NIAB-846-14	66	37.0	3.9	29.4	29.3	85.6
NIAB-846-16	52	38.5	4.6	35.1	28.3	86.4
NIAB-846-25	43	37.0	5.0	34.4	28.9	87.6

highest yield at 12 inches (30cm) spacing followed by 18 inches (45cm). These results indicated that NIAB-846 has wider adaptability to different plant to plant spacing with the field stability even at higher plant density. 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 12).

DISCUSSION

In different mutated generations the variations in different plant traits i.e. faster in growth, vigor etc reflects the applications of radiations affect. The mutated plants also showed resistance to old strain and tolerance to new Burewala strain of cotton leaf curl virus. It was noted that maximum variation was in M_2 generation plant progenies and some of the individual plants possessed desirable economic traits along with resistance/tolerance to CLCuV disease under high disease pressure and that were selected for further evaluation. These variations are may be due to genetic recombination/modifications in the chromosomes i.e. aberrations, deletions, insertions etc or even changes in the sequence of some genes. Such types of mutations/variations were earlier reported by different researchers in the plants. Various changes in leaf shape in cotton recorded by Muthusamy and Jayabalan (2000). Whereas; Muthusamy *et al*. (2005) selected high yielding mutants in cotton after irradiation treatments. Twin boll and some other boll abnormalities, morphological variations were observed in cotton mutant lines (Muthusamy *et al.* 2004; Muthusamy and Jayabalan, 2001).

Moreover, lower dose of gamma irradiation showed enhancing effects on growth of vegetative and reproductive parts of plants alongwith 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) which ultimately increases the growth and number of cells and the whole plant. In the present study, the pollen irradiation technique is found 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 are minimized. In seed irradiation whole genome is affected by irradiation, therefore a large M_2 population is required in seed irradiation (Iqbal *et al*., 1994). Whereas in the present study of pollen irradiation a small M_2 population was required and higher frequency of mutants was recorded. Earlier similar results were reported by (Jalil and Yamaguchi, 1965; Vig, 1973). Pollen irradiation before cross-pollination/hybridization created useful induced mutations in cotton. Such types of finding were 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 was also reported by different researchers (Aslam *et al*., 1994; Aslam and Stelly, 1994; Aslam, 2000; Aslam and Elahi, 2000; Aslam, 2002; Aslam *et al.,* 2009).

Due to irradiation effects, NIAB-846 exhibited on an average 18.9% higher yield than standard in various trials. NIAB-846 is also early maturing with desirable plant type. It has sympodial type plant with fruit bearing monopodia having desirable foliage and medium stature. It has better tolerance to CLCuV and 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.

***Conclusion*:** The mutant NIAB-846 has better plant type, early maturing, desirable foliage and high yield potential. It has better tolerance to cotton leaf curl virus (CLCuV) disease along with good fibre characteristic 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. In addition, the technique pollen irradiation is also proved to be economical and useful to create new genetic variability for cotton plant improvement

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