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COMPARISON AMONG DIFFERENT COTTON (*GOSSYPIUM HIRSUTUM* L.) GENOTYPES WITH RESPECT TO MORPHOLOGICAL, FIBRE QUALITY ATTRIBUTES AND EXPRESSION ANALYSIS OF *CRY1AC* GENE

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ABSTRACT

Cotton is a cash crop of Pakistan and it plays a key role in the economy of the country for earning of foreign exchange. American cotton (*Gossypium hirsutum* L.) is the highest fibre producing specie of the world. The present study was conducted to check the comparison of morphological data, fibre quality traits and expression analysis of *Cry1Ac* gene in 14 cotton genotypes including hybrid (FI), F2 and F4 stages progeny in comparison with check variety MNH-886 at National Agricultural Research Centre, Islamabad during the year 2015 and 2016. The results of morphological traits revealed that field grown cotton genotypes showed variation in morphological characters. The highest plant height was observed 141.6 cm in HSP 7-4(2). Similarly, both maximum number of bolls per plant 115.60 and boll weight 5.92 g was reported in cotton hybrid NIGAB-4 respectively. The best internodes distance 2.32 cm was recorded in B-318-A genotype. There were significant differences observed in morphological traits of cotton except some genotypes. The results of fibre quality traits illustrated that optimum fibre fineness 3.1 µg/inch was reported in cotton hybrid NIGAB-4. While the best results of fibre strength 28.6 g/tex was measured in HSP 7-4(2) and hybrid NIGAB-3, length 33 mm in NIGAB-4, uniformity ratio 49.4 % in NIGAB-4 and fibre elongation 12.9 % was recorded in B1-37 genotype respectively. Meanwhile different *Bt* genes in 14 cotton genotypes (*Cry1Ac, Cry2Ab and Cry1F* genes) were checked through immunostrip assay and hence only Mon531 event for *Cry1Ac* gene of 346 bp was confirmed after immunostrip and PCR analysis. Quantification of *Cry1Ac* toxin was performed through sandwich ELISA at 80 and 120 days after sowing of leaf and boll tissues of 14 cotton genotypes. The *Cry1Ac* toxin level of these genotypes is in the range of 0.27 to 3.67µg/g based on fresh weight tissues. From the results, it has been concluded that cotton hybrids have performed well as compared to other tested cotton genotypes.

Key words: American Cotton, Variation, Morphological and fibre quality traits. *Cry1Ac* toxin. ELISA.

INTRODUCTION

Cotton is a cash crop of Pakistan and it plays a key role in the economy of the country for earning of foreign exchange. A lot of people get benefits from cotton in garments manufacturing, textile industry, production of edible oil and dairy industry (Ali *et al*. 2011; Ahmad *et al*. 2009). The significance of American cotton (*Gossypium hirsutum* L.) is obvious from the fact that it is the highest fibre producing specie of the world (Dutt *et al*. 2004).

Variation among the existing germplasm has been a base for developing best genotypes and improvement of these germplasms (Li *et al*. 2008). A variety of plant breeding tools like using of exotic germplasm, crossing, selection and mutation can be used to boost the estimation of variability in germplasm that will facilitate the breeders to select the best parental lines which can produce different segregating population to get better progenies (Saravanan *et al*. 2006; Esmail *et al*. 2008). Best cotton cultivar development through crossing of diverse parental lines has been early studied (Punitha and Raveendran 2004). It has also been documented by numerous researchers in earlier studies that better performance in terms of yield of cotton hybrids over parents has been reported (Hassan and Khan 1986; Kalwar *et al*. 1992). Therefore, practical advancements are required to elevate the yield of cotton seed through constant selection of best yielding varieties having wide range of adaptation to climatic environments and location specific varietal selection (Ashokkumar and Ravikesavan 2011). A group of researchers had studied cotton diversity in various germplasms (Lounge *et al*. 2007). Diversity in cotton morphological traits has been studied that can lead to identify the phenotypic changes (Esmail *et al*. 2008). Hence the choice of best parental lines for prospective cotton breeding must be based on genetics other than geological diversity (Thiyagu *et al*. 2011). It has been reported that cotton cultivars varied significantly in terms of number of bolls per plant and lint manifestation (Wang *et al*. 2004), ginning outturn (GOT), Cotton yield (Arshad *et al*. 2003; Ali *et al*. 2005; Sezener

et al. 2006; Ehsan *et al*. 2008) sympodial (fruiting) branches, number of bolls and boll weight (Qayyum *et al*. 1992).

Cotton genotypes differ regarding to fiber quality (Mohammad 2001) and lint percentage. The best quality of fibre for a specific cotton genotype is a mixture of diverse traits like fineness, staple length, strength and uniformity with great importance (Poehlman and Sleper 1995; Ali *et al*. 2008). Globally, quality traits of fibre lay down the foundation for marketing and trade of cotton (Asif *et al*. 2008).

Bt cotton is used to control bollworm a major insect pest of cotton. To cultivate *Bt* cotton, a lot of positive changes have been occurred in terms of yield and reduction in pesticides use (Wu and Guo 2005). The effectiveness of *Bt* cotton is correlated with the expression level of insecticidal genes (Gutierrez *et al.,* 2006). The performance of *Bt* genes for controlling target insect pests fluctuates among cotton varieties (Adamczyk and Sumerford 2001) age of plant (Wan *et al.* 2005) different parts of plant (Abel and Adamczyk 2004) types of gene and various environmental factors (Gore and Adamczyk 2004; Jackson *et al.* 2004). Though, due to the constant production of minute quantity of *Bt* toxin in cotton, the pest could develop resistance and abolish the advantages of Bt cotton (Liang *et al.* 2000).

Keeping in view the facts related to the significance of *Bt* cotton in Pakistan, this study was designed to estimate all relevant necessary information of cotton morphology, fibre quality and expression analysis of *Cry1Ac* gene. This study has covered a lot of information of different cotton germplasm at distinct growth stages due to which cotton growers can get benefits according to their demands for specific interests. As cotton production can be increased if growers cultivate hybrid seed parallel to commercially approved cotton variety as well. It is also necessary that there should be moderate level of *Bt* toxin to maintain their active role in controlling insect pests in local cotton genotypes before their approval of commercialization for cotton growers in the country.

MATERIALS AND METHODS

Plant materials: The open field experiments were conducted at National Institute for Genomics and Advanced Biotechnology (NIGAB), National Agricultural Research Centre (NARC) Islamabad during the year 2015 and 2016. A total of fourteen cotton (*Gossypium hirsutum* L.) genotypes seeds of distinct progenies (F1, F2 and F4) were obtained from NIGAB project to evaluate differences in morphological and fibre quality traits with respect to check variety MNH-886. Two F1 hybrids namely NIGAB-3 and NIGAB-4 while that of F2 stage cotton genotypes include HSP 7-4(1), HSP 7-4(2) and HSP 7-4(3). Similarly, F4 stage genotypes include B-318-B, B-415, B1-124, B1-5689, B-216-A, B1-37, B-216-B and B-318-A respectively.

Field experiments: Cotton seeds of 14 genotypes were sown in May 2015 and May 2016. All the experiments were performed in triplicate having complete randomized block design. For each genotype a total of 20 plants were grown per replication by planting 4-5 seeds per dibble. After germination, only one plant was maintained per dibble and the remaining plants were removed. Under field conditions of cotton equal agronomic practices were applied as per recommendations to all genotypes. At the same time the required measures of plant protection against insects were applied. Morphological data was collected from ten random plants per genotype per replication.

Morphological traits: For all cotton genotypes at distinct stages of growth cycle i.e. (F1, F2, F4 and MNH-886) morphological data recorded were include; plant height, number of bolls per plant, boll weight and internodes distance.

Fibre quality traits: After harvesting, the cotton seeds were ginned at NIGAB and their lint was determined in Fiber Technology Laboratory, Ayub Agricultural Research Institute, Faisalabad through HVI (High Volume Instrument). The studied fibre quality traits were Staple length (mm), fiber fineness (micronaire), fiber strength (g/tax), fibre uniformity (ratio) and fibre elongation (%).

Immunostrip analysis: For immunostrip assay, 100 mg of fresh leaf samples from field grown 14 cotton genotypes were taken according to manufacturer's instructions (Agdia Inc. USA) for the detection of *Cry1Ac*, *Cry1F* and *Cry2Ab* genes. Care was taken to avoid the entrance of strips more than 0.5 cm or ¼ inch during the reaction time. The reaction was considered valid when control line was appeared within 3 min. Two types of immunostrip specific for "*Cry1F*" (Cata. #: STX010300) "*Cry1Ac and Cry2Ab*" (Cata. #: STX06800) genes were used. Reaction results were recorded as positive (+) and negative (-) based on test line appearance on the strip within due time.

DNA extraction and PCR analysis: DNA was extracted from the plants of each entry through CTAB method (Doyle and Doyle 1987). Fresh (100 mg) leaf sample was ground into fine powder through liquid nitrogen. Event specific primers (Mon 0531) and internal gene primer Sad1 were synthesized using the following sequences (Yang *et al*. 2005).

Mon531 F: AAGAGAAACCCCAATCATAAAA
Mon531R: GAGAATGCGGTAAAGATACGTC
Sad-1F: CCAAAGGAGGTGCCTGTTCA
Sad-1R: TTGAGGTGAGTCAGAATGTTGTTC

PCR was carried out using Veriti® thermal cycler (Applied Biosystems, USA). The reaction conditions were as follows; pre-denaturation 94°C for 5 minutes followed by 35 cycles each comprised of denaturation at 94°C for 1 minute, annealing at 57°C for 1 minute and extension at 72°C for 1 minute. Finally post extension was carried out at 72°C for 10 minutes. The product was separated on 1% agarose gel and amplified bands were visualized by ultra-violet trans illuminator. Amplicons size of 346 bp and 107 bp expected to be obtained for Mon531 and SadI respectively.

Sandwich-ELISA: At two growth stages i.e. 80 and 120 days after sowing (DAS) quantification of *Cry1Ac* toxin was carried out through sandwich-ELISA. Third fully expanding leaf and boll tissues of each genotype were used for analysis. Twenty milligram (20 mg) fresh plant tissues were sampled and grinded manually in extraction buffer as per manufacturer instruction (Envirologix Inc. USA). The optical density reading was measured on micro plate reader (Bio-Rad imark^{TM} USA) at 450 nm. Finally, toxin level (µg/g) was calculated by using simple regression analysis.

Statistical analysis: The data were subjected to excel sheets and their means were compared by using analysis of variance through statistical software 8.1. (Analytical Software, 2005).

RESULTS

The data of morphological parameters, fibre quality traits and expression analysis of Bt gene were recorded. The details of each entry are given below.

Plant height: The plant height at maturity was measured for all the selected genotypes of cotton. The variety MNH-886 showed an average of 128.80 cm plant height of ten random selected plants of three replications. In between the two hybrids, NIGAB-3 exhibited maximum mean plant height 138 cm as compared to NIGAB-4 (134.8 cm). The best mean plant height 125.40 cm was recorded in F4 population of B-318-B. Similarly, in F2 progenies the best result of plant height was measured 141cm in HSP 7-4(3). Statistical results of mean plant height represent significant differences in all genotypes except MNH-886, B-318-B, HSP 7-4(2) and B1-37 respectively as shown in table 1.

Number of bolls per plant: Mean No. of bolls per plant of 14 cotton genotypes were counted separately and they were compared with each other and MNH-886 for the best bolls production. From the results, variety MNH-886 produced 84.60 mean bolls per plant. Out of two F1 hybrids NIGAB-4 has produced maximum mean no. of bolls 115.60 per plant as compared to NIGAB-3 105.60 bolls per plant. There were significant differences observed in between the two hybrids of cotton. In F4 population the optimum mean no. of bolls per plant i.e. 91.80 was recorded in B-318-B while minimum no. of bolls 33.20 was noted in B-318-A genotype. In mean no. of bolls statistical differences were seen in F4 stage cotton genotypes. In F2 stage cotton genotypes, the highest no. of bolls per plant was observed 105.20 in HSP 7-4(3) while the lowest bolls 95.60 were seen in HSP 7-4(2) respectively. Statistically there were no significant differences in HSP 7-4(3) and HSP 7-4(1) genotypes in terms of bolls production while HSP 7-4(2) showed significant difference from the other two F2 progenies (as shown in table 1). The overall mean no. of bolls/ plants of all 14 genotypes were 72.63 and the best mean bolls 115.60 was observed in cotton hybrid NIGAB-4 as compared to all genotypes.

Internodes distance: Internode distances was measured for 14 cotton genotypes under field condition. In this experiment, ten randomly plants were selected per replication/genotype. From each selected plant of cotton five internodes distance were randomly measured per branch and then their mean was taken for analysis. Different plant stages of cotton exhibited different internodes distance. The results revealed that cotton check variety MNH-886 has 4.49 cm mean internodes distance. Significant differences were seen in mean internodes distance of NIGAB-3 and NIGAB-4. The results of F4 populations have represented that higher distance 3.42cm was observed in B-318-B. Some F4 genotypes were statistically different while other showed no any significant differences in mean internodes distance (see table 1). The mean distances of F2 population showed that there were no significant differences noted in all 3 genotypes. The overall average internodes distance of all 14 genotypes was reported as 3.72 cm.

Boll weight: Boll weights of all genotypes were taken at crop maturity i.e. at picking stage. Ten bolls per plant were taken from the ten random selected plants per replication and it was weighted separately through digital balance. The average boll weight was measured for each genotype as shown in table 1. The mean bolls weight of MNH-886 was 4.77 g. Similarly, the best bolls weight in hybrid NIGAB-4 was 5.92 g followed by NIGAB-3 (5.84 g) respectively. The boll weights of these two hybrids were statistically non- significant. The data of F4 generation showed that optimum mean bolls weight observed in B-216-B (4.87g) followed by 4.25g in B1-124 genotype. In most of the F4 generation there were significant statistical differences observed. The bolls weight of F2 lines revealed that HSP 7-4(2) and HSP 7-4(3) have the same weight 4.82g as compared to 3.82g in HSP 7-4(1) respectively. There were no significant differences in HSP 7-4(2) and HSP 7-4(3) while HSP 7-4(1) showed statistical difference in bolls weight.

Percentage wise comparison of morphological traits with cotton check variety MNH-886: Percentage wise comparison of morphological traits of all cotton genotypes were checked with control variety MNH-886. The comparative results of plant height at maturity demonstrated that both hybrids i.e. NIGAB-3 and NIGAB-4 have showed increase in plant heights 7.14% and 4.6% than MNH-886. In contrast to hybrids, F4 populations have showed decreased in % age of plant heights in comparison with cotton check variety. At the same time the results of F2 populations have drastically increased plant heights in the range of 2.63% to 9.47% from MHH-886.

No. of bolls per plant % age wise comparison represented that both hybrids NIGAB-3 and NIGAB-4 have increased 24.8% and 36% than check variety. While in F4 population, only one-line B-318-B showed 8.51% increase in mean no. of bolls per plant than MNH-886. The remaining lines have decreased mean no. of bolls per plant from -27 % to -60 % as compared to MNH-886. The results of F2 lines revealed that 13% to 24.34% increase observed in mean no. of bolls than MNH-886 (table 2).

Internodes distance of NIGAB-3 has increased 5.79% while that of NIGAB-4 decreased when compared with MNH-886. All the F4 genotypes have showed % age wise reduction in internodes distances in comparison with MNH-886. There was gradual increase appeared in HSP 7-4(1), 2 and 3 in internodes lengths from 10.2% to 22.7% as compared to cotton variety MNH-886.

Bolls weight of NIGAB-4 and NIGAB-3 were higher 24.1% and 22.4% than MNH-886. In F4 population of cotton the only line B-216-B has performed dominant boll weight 2.09 % while rest of all lines showed poor performance of boll weights (%) in comparison with Pakistani approved cotton variety MNH-886.

Fiber quality traits of cotton: The data regarding fiber quality traits of 14 cotton genotypes include fiber fineness, fiber strength, fiber length, fiber uniformity and fiber elongation were determined through HVI analysis.

Micronaire is a sign of air permeability and it is regarded as a signal for fineness and maturity of cotton fibre. The results of fiber fineness of MNH-886 showed 4.6 µg/inch. In comparison hybrids NIGAB-3 and NIGAB-4 obtained 3.3 µg/inch and 3.1 µg/inch fineness respectively. Fiber fineness of F4 and F2 cotton genotypes has considerable range of 3.2µg/inch to 5.8µg/inch (table 3).

Fiber strength is represented by the capability to resist being pulled apart when stresses occur. This character is also determined by the quality of cotton that should be of long staple and is highly twisted. In the present study, the fiber strength was determined for all the 14 genotypes of cotton. The variety MNH-886 proclaimed 28.3 g/tex fiber strength. On the other hand, hybrids NIGAB-3 and NIGAB-4 fabricated 28.6 g/tex and 27 g/tex strength respectively. The remaining populations (F4 and F2) fall in the range of 23.1- 28.6 g/tex of fiber strength measures.

Length is one of the most important properties of cotton fibers. Longer fibers are generally finer and stronger than shorter ones. The length traits of fiber are determined by genetic factors as well as by ginning and textile processing conditions. The overall length of fiber ranged from 24.7 mm to 33 mm. Hybrids showed maximum fiber length as compared to all other tested genotypes as given in table 3. HSP 7-4(1), 2 and 3 have appropriate lengths in the range of 27-30.9 mm. Variation was observed among the F4 stage cotton lines. Its length varies from 24.7 mm to 28.5 mm respectively. The cotton check variety exhibited fiber length 29.2 mm.

The maximum uniformity 49.4 % was obtained in NIGAB-4 followed by 48.8% in HSP 7-4(2). While other genotypes followed in the range of 41.3% to 48.7% respectively.

Fibre elongation is a key cotton fibre trait that directly affects yarn and fabric strength and extensibility. Fibre elongation measures the elasticity of fibers before a break occurs. Fibre elongation is expressed in percentage. The present results demonstrated that variety MNH-886 produced 10.9 % fiber elongation. The highest elongation was scored 12.9 % in B1-37 cotton genotype while the lowest fiber elongation 10% reported in B-318-A (shown in table 3).

ImmunoStrip analysis: Fourteen local *Bt* cotton genotypes were analyzed by immunostrip assay for the determination of types of commercial *Bt* genes (*Cry1Ac*, *Cry2Ab* and *Cry1F*). Result revealed that all 14 genotypes harbored*Cry1Ac* gene while immunostrip test for *Cry1F* (Wide Strike event) and *Cry2Ab* (Bollgard-II event) genes showed negative reactions for all the tested genotypes (table 4 and fig. 1).

PCR analysis: PCR test was performed for the confirmation of *Cry1Ac* gene. PCR result confirmed the existence of Mon531 event in all cotton genotypes. Amplicon size of 346 bp was obtained for all Mon531 positive genotypes. Along with Mon-531 event, cotton endogenous gene or internal control (*Sad1)* was also amplified in all genotypes with the product size of 107 bp as shown in (fig. 2). DNA ladder (1 kb) was used to confirm the approximate size of amplified products.

Quantification of *Cry1Ac* toxin in leaf and boll tissues at 80 DAS: Cotton genotypes that were considered as positive in immunostrip assay for Cry1Ac gene were further subjected to sandwich ELISA for quantification of Cry1Ac toxin. Leaf and bolls were sampled from each plant and were tested for Cry1Ac toxin through sandwich ELISA. From the results, the quantity of Cry1Ac toxin

level in leaf tissues on fresh weight basis of all the specified plants ranged from (0.562 to 3.676µg/g) at 80 DAS. The highest expression level of Cry1Ac was measured in the cotton hybrids NIGAB-4 and NGAB-3 as (3.67 and 2.93 µg/g) while moderate expression level was given by the genotype HSP7-4(3) i.e. 1.95 µg/g while the lowest expression level was recorded in the genotype B318A 0.562 µg/g respectively. Results of Cry1Ac toxin have shown variation among the tested genotypes. While in boll tissues, the quantity of *Bt* toxin in all 14 cotton genotypes on fresh weight basis at 80 DAS ranged from 0.48 to 3.30 µg/g. The highest expression level of Cry1Ac gene was measured in the hybrid NIGAB-4 (3.30 µg/g) while moderate expression level was reported in F2 stage progeny of cotton HSP7-4 (3) i.e. 1.62 µg/g and the lowest expression level was recorded in the genotype B318A (0.48µg/g) as shown in fig. 3.

Quantification of *Cry1Ac* toxin in leaf and boll tissues at 120 DAS: Leaf and boll tissue samples were taken from the same specified plants of 14 *Bt* cotton genotypes at 120 DAS. Results of *Bt* toxin level in leaf and boll tissues are presented in Fig. 4. The quantity of *Cry1Ac* toxin in leaf tissues on fresh weight basis ranged from (0.41 to 3.23 µg/g) among cotton genotypes. At 120 DAS, again NIGAB-4 has the highest expression level (3.23µg/g) while the lowest expression was recorded in the genotype B318A (0.41µg/g). Results clearly showed that all the *Bt* cotton genotypes vary with respect to *Bt* toxin levels. The quantity of *Bt* toxins in boll tissues on fresh weight basis ranged from (0.27 to 2.92 µg/g). In boll tissues, NIGAB-4 has the highest expression level (2.92µg/g) while the lowest expression was recorded in the genotype B318A (0.27µg/g). Based on overall results gradual reduction in *Cry1Ac* toxin level was observed when crop goes to maturity i.e. at 120 DAS in bolls as compared to 80 DAS and leaf stage respectively.

Table 1. Comparison of morphological diversity in cotton genotypes.

S. No.	Genotype	Plant height (cm)	No. of bolls/plant	Internode distance (cm)	Boll weight (g)
1	MNH-886	128.80^{abc}	84.60^{abc}	4.49^{ab}	4.77^{ab}
2	B-318-B	125.40^{abc}	91.800^{ab}	3.42^{bc}	3.46^{bc}
3	B-415	102.40^{cd}	50.40^{cd}	2.53^{c}	2.88^{c}
4	HSP 7-4(1)	136.60^{a}	102.80^{a}	5.51^{a}	3.82bc
5	HSP 7-4(2)	132.20^{abc}	95.60^{ab}	5.12^{a}	4.82^{ab}
6	HSP 7-4(3)	141.00^{a}	105.20^{a}	4.95^{a}	4.81^{ab}
7	B1-124	84.20^{f}	40.40^{d}	3.01^{c}	4.25^{abc}
8	B1-5689	99.80^{de}	45.00^{d}	2.76^{c}	3.77^{bc}
9	B-216-A	86.60^{ef}	50.20^{cd}	2.92^{c}	3.33^{bc}
10	B1-37	117.80^{abc}	61.40^{bcd}	2.53^{c}	3.95^{bc}
11	B-216-B	88.40^{ef}	35.00^{d}	3.23^{bc}	4.87^{ab}
12	B-318-A	103.80^{bc}	33.20^{d}	2.32^{c}	3.72^{bc}
13	NIGAB-3	138.0^{a}	105.60^{a}	4.75^{a}	5.84^{a}
14	NIGAB-4	134.8^{ab}	115.60^{a}	4.47^{ab}	5.92^{a}
	Standard error	± 6.313	± 11.06	± 0.36	± 0.49
	Overall mean	115.7	72.63	3.72	4.3

Mean values followed by a common letter in the respective column do not differ by LSD 0.05.

Table 2. Percentage (%) wise comparison of cotton genotypes with check variety MNH-886.

Genotype	Plant height (cm)	No. of bolls/plant	Internode distance (cm)	Boll weight (g)
B-318-B	-2.63975	8.510638	-23.8307	-27.4633
B-415	-20.4969	-40.4255	-43.6526	-39.6226
HSP 7-4(1)	6.055901	21.513	22.71715	-19.9161
HSP 7-4(2)	2.639752	13.00236	14.03118	1.048218
HSP 7-4(3)	9.47205	24.34988	10.24499	0.838574
B1-124	-34.6273	-52.2459	-32.9621	-10.9015
B1-5689	-22.5155	-46.8085	-38.5301	-20.9644
B-216-A	-32.764	-40.6619	-34.9666	-30.1887
B1-37	-8.54037	-27.4232	-43.6526	-17.1908
B-216-B	-31.3665	-58.6288	-28.0624	2.096436
B-318-A	-19.4099	-60.7565	-48.3296	-22.0126
NIGAB-3	7.142857	24.8227	5.790646	22.43187
NIGAB-4	4.658385	36.64303	-0.44543	24.10901

Table 3. Comparison of Fiber Quality Traits in Cotton Genotypes.

Genotype	Fiber fineness(µg/inch)	Fiber strength (g/tex)	Fiber length (mm)	Uniformity ratio (%)	Fiber elongation (%)
B-318-B	3.5	23.1	24.7	44.6	11.8
B-415	3.2	25.9	27.6	42.9	11.6
HSP 7-4 (1)	5.8	24.5	27	47.8	11.8
HSP 7-4 (2)	4.9	28.6	30.9	48.8	10.4
HSP 7-4 (3)	4.8	24	27.8	45.8	12.7
B1-124	4.7	23.2	28.1	41.3	12.2
B1-5689	5.1	24.2	27.6	43.8	11.7
B-216-A	5.4	24.2	27.8	44.5	12.2
B1-37	4.5	24.6	28.5	41.6	12.9
B-216-B	4.7	23.5	26.3	43.3	11.5
B-318-A	5.0	23.1	27	44.8	10
NIGAB-3	3.3	28.6	32.7	49	12
NIGAB-4	3.1	27	33	49.4	12.7
MNH-886	4.6	28.3	29.2	47.7	10.9

Table 4. Immunostrip assay for 14 local *Bt* cotton genotypes.

Genotype	*Cry1Ac*	*Cry2Ab/1F*	Genotype	*Cry1Ac*	*Cry2Ab/1F*
MNH 886	+	-	B-415	+	-
NIGAB-4	+	-	B1-124	+	-
NIGAB-3	+	-	B1-5689	+	-
HSP 7-4(1)	+	-	B 216A	+	-
HSP 7-4(2)	+	-	B1-37	+	-
HSP 7-4(3)	+	-	B216B	+	-
B-318 B	+	-	B318A	+	-

(+) = *Bt* gene presence, (-) = *Bt* gene absence

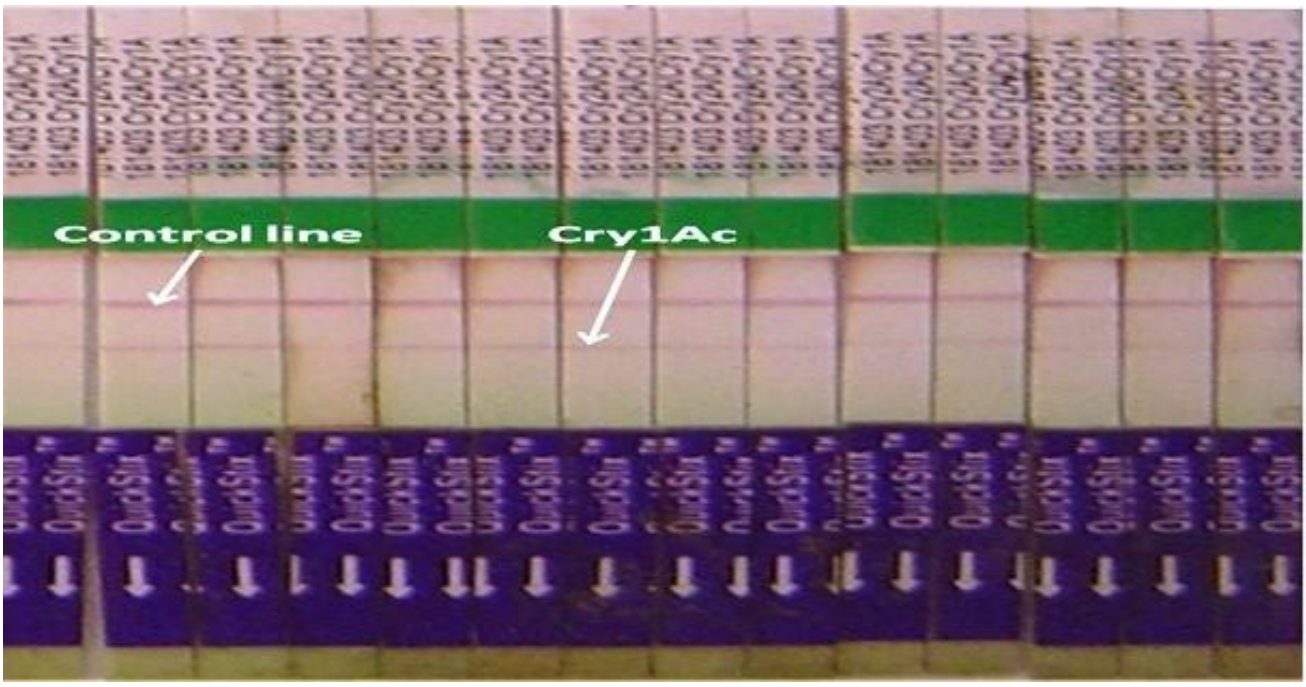

Fig.1. Imuunostrip test of 14 cotton genotypes

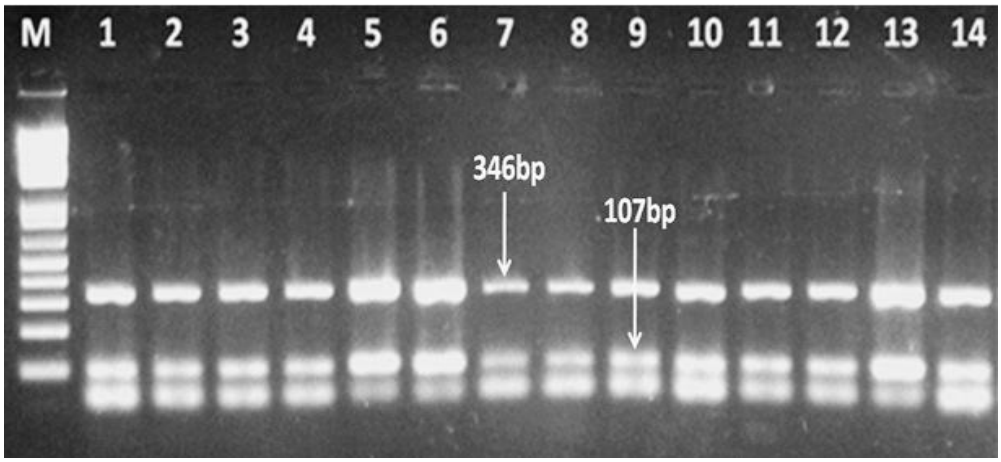

Fig. 2. Confirmation of *Cry1Ac* gene (Mon531 event) in 14 *Bt*-cotton genotypes through PCR

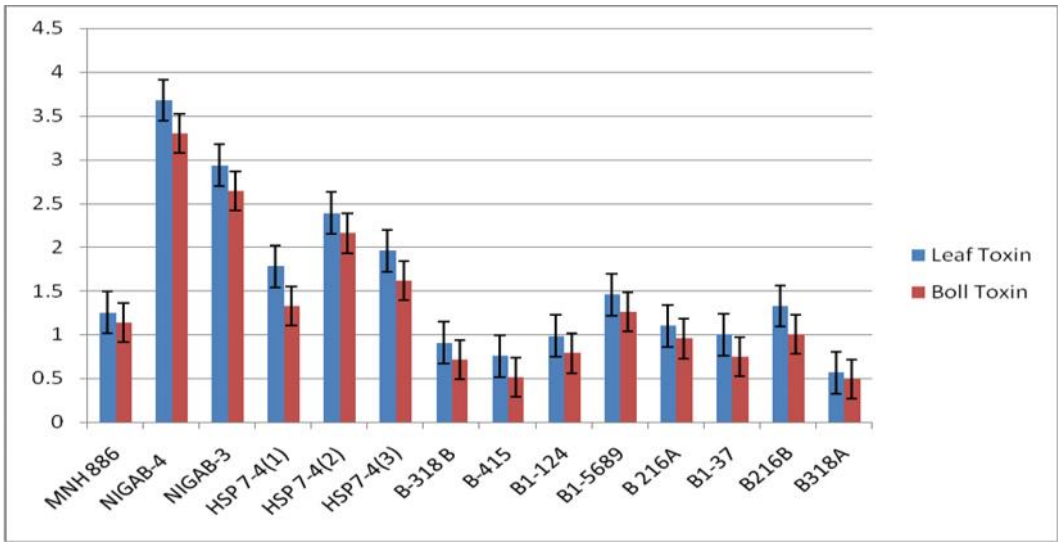

Fig. 3. Quantification of *Cry1Ac* toxin (µg/g) in leaf and boll tissues at 80 DAS

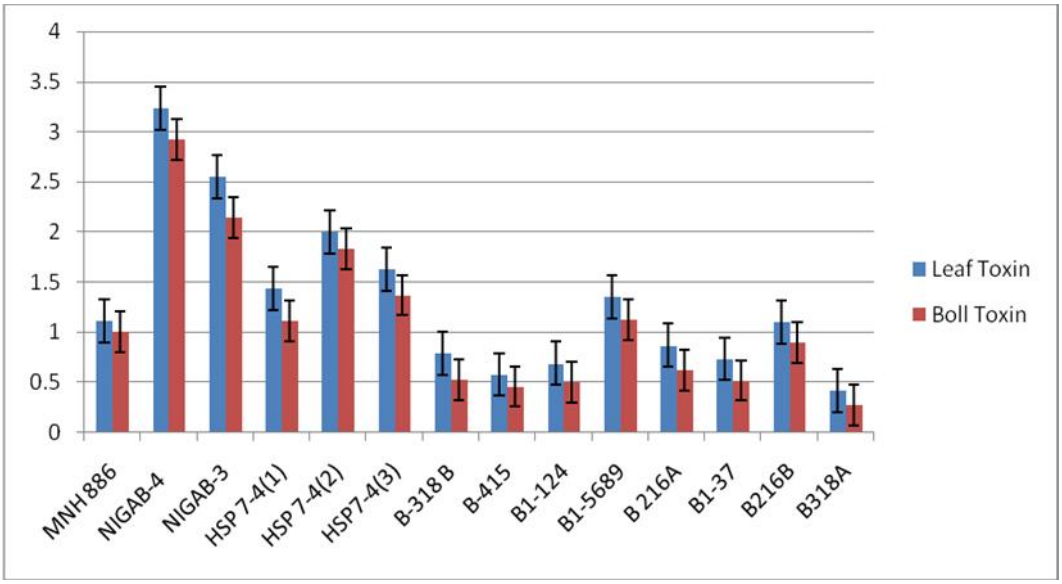

Fig. 4. Quantification of *Cry1Ac* toxin (µg/g) in leaf and boll tissues at 120 DAS

DISCUSSION

In the present study, different morphological traits of cotton plants were studied. The morphological results revealed variations in each trait and genotype. Same to our findings of useful morphological diversities among cotton genotypes were also reported by other researchers (Ashokkumar and Ravikesavan 2011; Malik *et al*. 2011). As it has been find out by other researchers that transgenic cotton crop has unintended effects on phenology of plant due to the changes in nutritional effects inside soil condition (Cellini *et al*. 2004; Pons *et al*. 2012). All the fourteen cotton genotypes varied significantly for their plant height. The maximum plant height (141cm) was measured in HSP 7-4(3) and the minimum 84.20 cm was reported in B1-124. The disparities seen for plant height among cotton genotypes can be ascribed to genetic variation in cotton plants. Our results of plant height are parallel to the findings of other scientists (Killi *et al*. 2005; Wankhade and Gobble 2002; Anwar *et al*. 2002; Copur 2006) who studied significant differences among cotton genotypes.

In the present study, the best bolls per plant (115.60) was observed in cotton hybrid NIGAB-4 while the lowest 33.20 bolls reported in B-318-A. This is supported by (Hussain *et al*. 2007) who observed number of bolls per plant 32.20 in cotton variety CIM-473. In mean no. of bolls there were statistical differences observed among the cotton genotypes. Similar to these results (Ehsan *et al*. 2008; Saeed *et al*. 1996) also reported that the no. of bolls varies significantly in different genotypes of cotton.

The results of internodes distance revealed that cotton check variety MNH-886 has produced 4.49 cm mean internodes distance. Internodes distance was in the range of 2.32 cm to 5.5 cm while the overall mean of 14 genotypes was 3.72 cm. Our results are divergent with that of Batool *et al*. (2010) who find out that internodes length was ranged from 2.12 to 2.47 cm among the tested cotton genotypes. These differences might be due to the differences in genotypes, environmental conditions and nutrients application.

Boll weight was measured for all cotton genotypes. Variation observed in bolls weight ranging from 2.8 - 5.92 g in cotton genotypes. The overall mean boll weight of 14 genotypes was 4.3 g. According to the published information of Ahmad *et al*. (2008); Khan *et al*. (2009a); Hofs *et al*. (2006) that differences in boll weight among upland cotton genotypes were recorded.

Different fiber quality traits were examined in this study. Data of five fiber traits i.e. fibre fineness, strength, length, elongation and uniformity ratio of the selected cotton genotypes were screened through HVI equipment. All the tested fibre qualities were higher in hybrids as compared to other genotypes and among the rest genotypes considerable variation exists in fibre traits. The outcome of heterosis are in accordance with that of Tuteja *et al*. (2005). Fiber properties differ as a task of the different genotypes and may also be due to the cultural practices and environment.

The results of fiber fineness described that it ranged from 3.1-5.1 µg/inch in all 14 genotypes. NIGAB-4 exhibited best fineness as compared to others. Similarly, maximum fiber strength 28.6 g/tex and length 33 mm was recorded in NIGAB-3 and NIGAB-4 respectively. The present results suggested that fibre fineness, strength and length have direct impact on each other. Other investigators also reported similar results (Ulloa and Meredith 2000; Zhang *et al*. 2005).

Other fibre quality traits like uniformity and elongation were also studied. Length uniformity is the ratio between the mean length and the upper half mean length of cotton fibers within a sample. It is well known that higher the fibre length % age, the greater will be the uniformity. The best uniformity ratio 49.4 % was recorded in NIGAB-4. The optimum fiber elongation 12.9 % was secured in B1-37 genotype. The same results i.e. 49 % uniformity ratio was observed by Jyotiba *et al*. (2010) in two hybrids of cotton. Contradictory to our findings Alikhasi *et al*. (2012) reported 8.16 % fibre elongation ratio in Mehr variety of cotton. Ashokkumar and Ravikesavan (2011) also reported 9.6 % elongation ratio. These differences may be due to different genotypes, agronomic practices and environmental conditions. Suinaga *et al*. (2006) and Meena *et al*. (2007 also reported about cotton plant phenology and other fibre related traits that variations have been noted in diverse germplasm of upland cotton.

Determination of type and number of different *Bt* genes was essential prior to quantification of toxin level. In the current study, the entire tested genotypes were positive having only *Cry1Ac* gene while other genes *Cry1F* and *Cry2Ab* were absent through immunostrip analysis. Additional confirmation regarding the existence of non-patented event Mon531 for *Cry1Ac* gene was performed through PCR analysis. Similar results were also reported by Ali *et al*. (2010) regarding the existence of *Cry1Ac* gene in 36 *Bt* cotton genotypes in Pakistan. They also confirmed Mon531 event for *Cry1Ac* gene through PCR. Event Mon531 is specific for *Cry1Ac* gene only (Yang *et al*. 2005). Same to our results of *Bt* genes type and number (*Cry1Ac* and Mon531 event of 346 bp amplicon size) were also confirmed by Iqbal *et al*. (2013) in Pakistani ten Bt cotton genotypes.

After confirmation of *Cry1Ac* gene through immune Strip and PCR analysis we have further subjected the expression analysis of *Cry1Ac* gene through sandwich ELISA. In the present study the quantification of *Cry1Ac* toxin was carried out at two growth stages and two tissues to compare the toxin level in all 14 cotton genotypes. The toxin level at 80 DAS in leaf samples of all genotypes ranged 0.562 to 3.676µg/g while that of

boll tissues fall in the range of 0.48 to 3.30 µg/g respectively. According to USDA 1.5 µg/g of *Bt* toxin is mandatory for durable pest resistance. Similarly, at 120 DAS the leaf and bolls toxins are in the range of 0.41 to 3.23 µg/g and 0.27 to 2.92 µg/g respectively. Leaf tissue has higher No. of *Cry1Ac* toxin as compared to boll tissues. Variation in *Cry1Ac* toxin was observed among the 14 cotton genotypes at different plant parts and growth stages as well. Similar to our findings Kranthi *et al*. (2005) also studied that leaf has higher toxin than other plant parts. Variation in expression of Bt gene occurs due to its base sequences and copy number, used promoter, and gene incorporation point into the DNA of target Bt varieties (Guo *et al*. 2001; Rao 2005). Adamczyk and Sumerford (2001) reported that toxin levels are higher at early developmental stages of crops but at maturity stage toxin level decreased. From this study it has been inferred that two hybrids NIGAB3 and NIGAB 4 were the most capable genotypes that produced almost outstanding performance in cotton phenology and other studied traits. So, this can be recommended that hybrid can perform well and it is need of the day to produce more hybrid seeds for better production and yield related attributes of cotton. Hence these genotypes can also be used in further breeding centers.

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