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Utilization of Natural and Genetically-engineered Sources in *Gossypium hirsutum* for the Development of Tolerance against Cotton Leaf Curl Disease and Fiber Characteristics

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ABSTRACT

The present study efforts have been made to combine natural and genetically-engineered resistance to get enhanced tolerance against cotton leaf curl disease (CLCuD) and improvement in fiber characteristics. Maximum number of tolerant plant against CLCuD was observed in the families of NIBGE-115 × transgenic Coker-312 expressing antisense rep, whilst minimum number of plants was in the families of FH-1000 × transgenic rep Coker-312 cotton. It was noted that ginning out turn; fiber fineness was significantly increased in F_1 and F_2 of NIBGE-115 × transgenic antisense rep Coker-312. Significant increase for fiber length was observed in the families of CIM-496 × transgenic antisense Coker-312 but non-significant differences were observed in all of the families of the crosses. The positive and highly significant correlation coefficient was observed between fiber length and fiber strength. The sample of parent plant material was small in the present study and did not represent the whole of the germplasm of *G. hirsutum,* therefore it would be worth-while to conduct another experiment involving large number of parents from the germplasm in a crossing program to substantiate the present findings. © 2010 Friends Science Publishers

Key Words: Genetically-engineered; *Gossypium hirsitum*; Natural sources; Introgression; CLCuD; Fiber

INTRODUCTION

Breeding of crop plants for the development of disease resistance is an important activity of improvement programs. These activities help the development of varieties with improvement of built-in genetic resistance to diseases, help protect environment, boost agricultural productivity and make crop production a profitable venture. Crop plants have a range of genes conferring resistance against number of variants that are referred to as biotypes or races and concerned host. These genes are generally present in different genotypes but need to be brought into the commercial varieties for effective disease management (Kumaran, 2005). Most of the crop plants suffer from several diseases in a particular agro-climatic or cultural condition. This situation provokes the plant breeders to develop a commercial variety or F_1 hybrids that has all the required resistance genes conferring resistance to all the prevailing races of the diseases affecting the species in a particular zone/cultural condition. This objective can be achieved by employing suitable breeding procedures like backcross breeding methods and using donors for various genes in tandem or convergent crossing program, incorporating all the desired genes into the common recipient parent.

Breeders are developing cotton varieties either by introduction or by hybridization for high yield as well as for improved fiber quality characters like fiber yield and fiber quality characters including fiber length, fiber strength and fiber fineness etc., according to the demand of consumers. These quality characters must be present in disease-resistant cotton plant. Several strategies for the development of cotton varieties with enhanced tolerance to cotton leaf curl disease (CLCuD) are being developed by the incorporation of different available sources of resistance in breeding programs. However, no effort has been made to pyramid multiple sources of resistance. Pyramiding of different disease resistance genes has been utilized for the development of bacterial blight resistant rice (Jiang, 2004). Most of the developed wheat varieties were based on few major genes responsible for resistance that leads to the adoption of mono-culturing regarding resistance genes. Same strategy was used for the development of wheat variety Inqilab-91 in Pakistan and PBW-343 in India, both were based on Yr27 gene (Singh, 2004), which has been broken down causing severe losses (Kisana, 2003). Thus, resistance based on such major genes has been lost very rapidly due to evolution of virulence by the pathogen to

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these genes. A reliable and alternative approach is to search for partial resistance based on minor genes, which impart durability. This type of resistance developed by recombination of minor genes is able to avoid rapid evolution of the rust pathogen of wheat to acquire new virulence. The exciting developments in genetically-engineered resistance against plant pathogens have provided new avenues for provisions of novel sources of resistance. The introgression among natural resistance sources and genetically-engineered resistance is an attractive possibility. Indeed, introgression of engineered and natural resistance was carried out for the development of broad-based resistance against tospoviruses, which is one of the limiting factors in yield losses worldwide (Gubba *et al*., 2002).

Begomoviruses are one of the most devastating pathogens of modern agriculture. Yield of cotton and several other crops suffer heavy losses due to emergence of resistance breaking strains (Choi *et al*., 2005), as CLCuD is one of the serious problem in Indo-Pak subcontinent with typical symptoms of vein darkening, leaf curling and vein swelling (Briddon *et al*., 2001; Qazi *et al*., 2007). The present experiments were conducted to see if diverse sources of resistance can be brought together. Several natural sources of resistance/tolerance against CLCuD are available like, NIBGE-115, MNH-770 and MNH-786; and engineered resistance is based on RNAi silencing like anti-sense tRep was also achieved against CLCuD. There are synthetic sources, which were brought from USA and Sudan and introduced in Pakistan but, unfortunately, showed susceptibility to CLCuD on their introduction.

The CLCuD is a noxipous disease of cotton, but different cotton varieties show tolerance against it. It was hypothesized that the combination of transgenic and natural tolerance will give additive and cumulative effects in proceeding populations with some levels of tolerance against CLCuD and also with enhanced fiber characteristics. The objectives were to explore the potential of genetically engineered and natural CLCuD tolerant cotton varieties.

MATERIALS AND METHODS

In order to investigate the pattern of disease tolerance against CLCuD in *G. hirsutum*, the experimental material for the studies was developed by crossing two female parents namely NIBGE-115 and FH-1000 with one pollen parent, transgenic Coker-312 (anti-sense tRep was used as transgene). The seeds of the parents were sown in 30 × 30 cm earthen pots using the glasshouse facility at National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan. The ambient temperature of the greenhouse during the November, 2005 was maintained between 20°C to 32°C by lighting mercury vapor lamps for the rapid growth and development of the plants. At the time of flowering, plants were emasculated and pollinated. All the necessary precautionary measures were taken to avoid alien pollen contamination of the genetic material at the time of emasculation and pollination. Maximum numbers of pollinations were made to produce sufficient number of F_1 seeds.

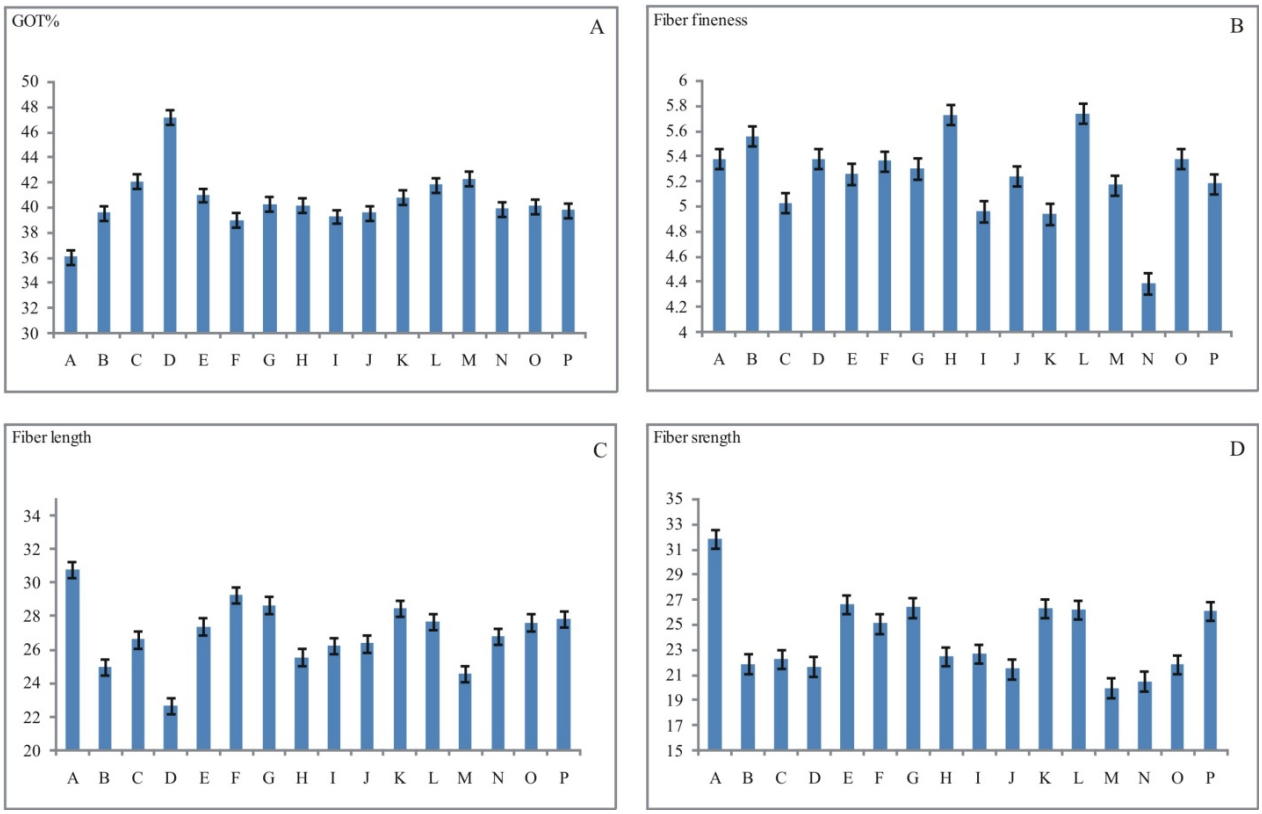
The crosses attempted in the greenhouse were: NIBGE-115 × Transgenic Coker-312, MNH-770 × Transgenic Coker-312, MNH-786 × Transgenic Coker-312, CIM-496 × Transgenic Coker-312, FH-1000 × Transgenic Coker-312. The F_1 seed and of six parents were planted at cotton research farm at NIBGE, Faisalabad, on 10th June, 2006. Each entry was sown in three replications following complete randomized block design to get unbiased observations. The seed was dibbled to ensure uniform plant population. The seed was sown in space of 30 cm within the row and 75 cm between the rows. Normal agronomic practices and plant protection measures were adopted during whole of the growing period. The F_2 seeds from individual plant were collected in bags. The F_2 seed of each plant was planted on 7th June, 2007 in three replications using complete randomized complete block design. Same plantation methodology was used to raise F_2 seeds as used for the plantation of F_1 seeds in field conditions. The visual observations were made on CLCuD symptoms on F_2 generation of each cross. A lint sample of 50 g was taken from all the plants and fiber length, fiber fineness and fiber strength for each plant were measured using Spinlab High Volume Instrument (HVI) in the NIBGE, Faisalabad. As HVI is fully computerized and measure all of fiber characteristics in a short time. Means of parents, F_1 and F_2 families for fiber characteristics were used for analysis. Pearson's correlation (r) coefficient (Steel & Torrie, 1997) was estimated among ginning outturn and fiber characteristics like fiber fineness, fiber strength and fiber length.

RESULTS

Assessment of CLCuD incidence in F_1 and F_2 generations: Incidence of CLCuD was assessed by visual observation on typical symptoms like vein clearing, vein swelling, leaf curling and leaf enations in F_1 and F_2 generations of NIBGE-115 × Transgenic Coker-312, MNH-770 × Transgenic Coker-312, MNH-786 × Transgenic Coker-312, CIM-496 × Transgenic Coker-312 and FH-1000 × Transgenic Coker-312. One plants out of thirteen was found to be infected in a cross of NIBGE-115 × Transgenic Coker-312; one plant out of 10 was found to be infected with CLCuD in a F_1 generation of MNH-770 × Transgenic Coker-312, three out of 13 showed typical symptoms of CLCuD in a cross of MNH-786 × Transgenic Coker-312, whereas two of six in each of the F_1 generation was found to be infected with CLCuD in a cross of CIM-496 × Transgenic Coker-312 and FH-1000 × Transgenic Coker-312.

The observations were made on varying number of plants in each family like F_2 generation of NIBGE-115 ×

Fig. 1: Comparison of fiber yield and other fiber characteristics of F_1 and F_2 generations. Panel A (Ginning out turn percentage), Panel B (Fiber fineness), Panel C (Fiber length) and Panel D (Fiber strength). A (NIBGE-115), B (MNH-770), C (MNH-786), D (CIM-496), E (FH-1000), F (Transgenic Coker-312), G (F_1 of NIBGE-115 × Coker-312), H (F_1 of MNH- 770 × Transgenic Coker-312), I (F_1 of MNH-786 × Transgenic Coker-312), J (F_1 of CIM-496 × Transgenic Coker-312), K (F_1 of FH-1000 × Transgenic Coker-312), L (F_2 of NIBGE-115 × Transgenic Coker-312), M (F_2 of MNH-770 × Transgenic Coker-312), N (F_2 of MNH786 × Transgenic Coker-312), O (F_2 of CIM-496 × Transgenic Coker-312), P (F_2 of FH-1000 × Transgenic Coker-312)



Transgenic Coker-312, MNH-770 × Transgenic Coker-312, MNH-786 × Transgenic Coker-312, CIM-496 × Transgenic Coker-312 and FH-1000 × Transgenic Coker-312 had 34, 33, 39, 50 and 49 number of plants, respectively. Thirteen plants out of 34 showed typical symptoms of CLCuD in F_2 of NIBGE-115 × Transgenic Coker-312. Seventeen plants were found to be symptomatic with CLCuD in MNH-770 × Transgenic Coker-312, MNH-786 × Transgenic Coker-312 and CIM-496 × Transgenic Coker-312 out of 33, 39 and 50, respectively whereas highest number of plants i.e., 36 out of 49 plants were found to be infected in the F_2 generation of FH-1000 × Transgenic Coker-312. This data showed maximum number of tolerant plants against CLCuD was present in a cross of NIBGE-115 × Transgenic Coker-312, whilst minimum number of tolerant plants were obtained from a cross of FH-1000 × Transgenic Coker-312.

Assessment of Ginning Outturn and Fiber Characteristics

Ginning outturn (GOT): The GOT was significantly ($P<0.05$) increased in F_1 of NIBGE-115 × Transgenic Coker-312 as compared to NIBGE-115 i.e., 36.1 to 40.3% and significant increased for GOT has also been observed from F_1 to F_2 generation of NIBGE-115 × Transgenic Coker-312 i.e., 40.3 to 41.8. Non-significant ($P>0.05$) increase in GOT was observed in MNH-770 and F_1 generation of MNH-770 × Transgenic Coker-312 indicated a non-significant increase in GOT but it was significantly improved (40.2-42.3) in F_2 generation. Ginning outturn of CIM-496 was significantly decreased in its F_1 and F_2 generations of CIM-496 × Transgenic Coker-312 like 47.2 to 39.6 and 40.1, respectively (Fig. 1A).

Fiber fineness (micronaire value): The difference in fiber fineness of NIBGE-115 was non-significantly ($P>0.05$) different from F_1 generation of NIBGE-115 × Transgenic Coker-312 (5.35-5.3) but significantly increased (i.e., 7.74) in case of F_2 generation. Non-significant ($P>0.05$) differences were observed in MNH-770 and F_1 generation (5.56 & 5.73) but were decreased significantly ($P<0.05$) in case of its F_2 generation (from 5.56 to 5.17). Similar observations were recorded, where non-significant

differences were recorded in CIM-786 and F_1 generation CIM-786 × Transgenic Coker-312 i.e., 5.03 and 4.96, respectively but decreased significantly in case of F_2 generation of CIM-786 × Transgenic Coker-312 i.e., 4.39, whereas non-significant (P>0.05) difference were noted in parent FH-1000 and its F_2 generation when cross was attempted with Transgenic Coker-312 such as 5.26 and 5.18, respectively (Fig. 1B).

Fiber length: Fiber length is one of the important fiber characteristics, which were determined in all the parents including its generations. Non-significant (P>0.05) differences were present in F_1 and F_2 generations of NIBGE-115 × Transgenic Coker-312 i.e., 28.67 and 27.71, which is significantly decreased from parent NIBGE-115 (30.8). Likewise non-significant (P>0.05) differences were observed in parent MNH-770 and its F_1 and F_2 generation when cross was attempted with transgenic Coker-312 such as 25.04, 25.58 and 24.57, respectively. It means that transgenic Coker-312 did not participate for the improvement of fiber length. Non-significant (P>0.05) differences were observed in parent MNH-786, F_1 and F_2 generations of MNH-786 × Transgenic Coker-312, which was 26.62, 26.25 and 26.81, respectively. Significant (P<0.05) improvement in fiber length was recorded from CIM-496 to F_1 and F_2 of CIM-96 × Transgenic Coker-312 (22.76 to 26.38 & 27.64, respectively). Non-significant (P>0.05) differences were observed FH-1000 and its F_2 generation (Fig. 1C).

Fiber strength: The F_1 and F_2 generations of NIBGE-115 × Transgenic Coker-312 showed significant (P<0.05) decrease in fiber strength to their parent NIBGE-115 i.e., 26.4 and 26.2 from 31.38 (Fig. 1D). Non-significant (P>0.05) differences were observed in MNH-770 and F_1 and F_2 of MNH-770 × Transgenic Coker-312 i.e., 21.9, 22.5 and 20, respectively. Non-significant (P>0.05) differences were also observed in MNH-786 and F_1 of MNH-786 × Transgenic Coker-312; values of fiber strength were 22.3 and 22.7 but reduced significantly in case of F_2 generation (20.5) as compared to parents and F_1 generation. Non-significant differences were also observed in case of CIM-496 along with F_1 and F_2 generations of CIM-496 × Transgenic Coker-312, value of fiber strength were 21.7, 21.5 and 20.5, respectively. Similar non-significant (P>0.05) results were found when fiber strength of FH-1000, F_1 and F_2 generation were recorded. Fiber strength determined by HVI was 26.6, 26.3 and 26.1, respectively (Fig. 1D).

DISCUSSION

In the present study, efforts have been made to recombine two different sources i.e., genetically-engineered resistance and natural sources of resistance against CLCuD. No report is available in literature about the introgression of different sources of resistance in cotton with the hypothesis that additive or cumulative effects may appear in subsequent generations. However, information is available in tomato against tospoviruses (Gubba *et al*., 2002) and potato tuberworm (Cooper *et al*., 2009) same approach have also been used for the control of Colorado potato beetle, which is a destructive pest of cultivated potato (Cooper *et al*., 2004). We used this information in *G. hirsutum* for the development of tolerance to CLCuD by the introgression of transgenic anti-sense tRep coker-312. These transgenic Coker-312 cotton plants showed tolerance to CLCuD in controlled conditions in containments when challenged with whiteflies collected from cotton field and also showed resistance in field conditions. The varying number of plants from F_1 and F_2 generations showed typical symptoms of CLCuD. These filial generations were developed by crossing five different natural tolerant cotton varieties and crosses with genetically engineered resistant cotton (anti-sense tRep Coker-312).

If we examine the number of infected plants in F_1 and F_2 generations from a cross of NIBGE-115 × Transgenic Coker-312, we found only one plant out of thirteen and 13 out of 34 from F_1 and F_2 respectively showed typical symptoms of CLCuD. More infected plants were observed in a cross of FH-1000 × Transgenic Coker-312 i.e., two out of six and 36 out of 49 from F_1 and F_2 generations, respectively. This data showed that transgenic Coker-312 contributed for enhanced resistance when this was recombined with NIBGE-115 but did not contribute with FH-1000 and similarly with MNH-770, MNH-786 and CIM-496 that's why more infected plants were observed in F_2 generation. These observations showed that the transgenic Coker-312 has good specific combining ability with NIBGE-115, suggesting dominant gene are involved in the inheritance of resistance of CLCuD (Ahuja *et al*., 2006) when crosses with NIBGE-115. Transgenic Coker-312 showed poor specific combining ability with MNH-770, MNH-786 and CIM-496. Although number of plant in this study are small but the information can be utilized to conduct this experiment on large area.

Cotton variety should have high GOT percentage with good fiber characteristics like fiber fineness, fiber length and fiber strength. Significant improvement for GOT has been noted from MNH-770 to F2 generation of MNH-770 × Transgenic Coker-312 (Fig. 1) but non-significant (P>0.05) the improvement of this combination were observed for fiber fineness, fiber length and fiber strength. Although the maximum numbers of infected plants with CLCuD were observed in combination of CIM-496 with Transgenic Coker-312 but showed significant improvement for fiber length. On the basis of overall results, NIBGE115 × Transgenic Coker-312 proved to be a good combination for the development of lines with improved CLCuD tolerance, GOT and fiber fineness but not good for fiber length and fiber strength, which could be recovered by backcrosses with NIBGE-115. No reports are available to support these finding but reports are available for the utilization of transgenic plants in introgression of tomato lines (Gubba *et al*., 2002). In the present study we were able to find best combination with respect to disease resistance, GOT, fiber

Table I: Correlation coefficient (r) between selected fiber characteristics in *Gossypium hirsutum*

	Fiber strength	Fiber length	GOT
Fiber fineness	0.239^{NS}	-0.048^{NS}	0.055^{NS}
Fiber strength		0.789^{**}	-0.462^{NS}
Fiber length			-0.738^{**}

** Significant at P<0.01
NS Non-significant, P>0.05

fineness and fiber length. We can utilize this information to design such experiments on large scale for the development of cotton varieties with improvement in yield contents and fiber characteristics. After the development of virus tolerant line, other desirable traits can be recovered by backcrossing (Laughlin *et al*., 2009), as we can use this approach in the cross of NIBGE-115 × Transgenic Coker-312.

Pearson's Correlation (r) coefficient revealed positive but were non-significant (P>0.05) correlation between fiber fineness and fiber strength (Table I), as reported for fiber fineness and GOT (Malik *et al*., 2009). Positive but non-significant correlation coefficient (P>0.05) were observed between fiber fineness and GOT. The correlation between fiber strength and fiber length was found to be positive and highly significant (P<0.01) (Asif *et al*., 2008). Negative and highly significant (P<0.01) correlation coefficient was found between fiber length and GOT (Azhar *et al*., 2004).

Our results showed the potential for broad resistance to CLCuD by combining engineered and natural resistance in a single plant and it seems that F_2 population is amenable to selection and single plant selection is effective in improvement in disease resistance, GOT and fiber characteristics. In the present study, the sample of parent plant material was small and did not represent the whole of the germplasm of *G. hirsutum.* Therefore, it would be worthwhile to conduct further experiment involving large number of parents from the germplasm in a crossing program to substantiate the present finding.

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