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Development of cotton leaf curls virus tolerance varieties through interspecific hybridization

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Cotton Leaf Curl Virus (CLCuV) is a major threat in enhancing cotton production in Pakistan. Economic loss due to this disease during last decade is estimated about 75 million rupees. This disease spread in epidemic from 1992 to 1995. The first CLCuV resistant variety was evolved in 1996. Resurgence of this disease occurred in 2001 (Burewala) breaking resistance of all exiting available germplasm of cotton. Interspecific hybridization for leaf curl virus resistance is the only economical and long term approach to tackle this hazardous problem. A total of 3338 genotypes were screened at Cotton Research station Vehari during 2003 to 2004 but none of these genotypes showed resistance to this disease. Two cultivated diploid species viz *Gossypium herbaceum* A1, *Gossypium arboreum* A2, *Gossypium anomalum* B1, *Gossypium capitiviridis* B4, *Gossypium gossypoides* D6, *Gossypium laxum* D8, *Gossypium stocksii* E1, *Gossypium somalense* E2, *Gossypium areysianum* E3 and *Gossypium longicalyx* x F1 did not showed the symptoms of this disease through petiole grafting. *G. arboreum* is immune to CLCuV, two artificial allotetraploids of 2(*Gossypium hirsutum* L x *Gossypium. anomalum*).x ³*G. hirs.* and 2 (*G. arboreum* L x *G. anomalum*). x ²*G. hirs.* were manually hybridized under field conditions. These two hybrids were also crossed for gene pyramiding [{.2 (*hirs.* x *G. anom.*) x ³*G. hirs.*} x {²*G. hirs.* x 2 (*G. arbo.* x *G. anom*) x ²*G. hirs.*}] x ²*G. hirs.* Exogenous hormones containing 50 mg/l gibberellic acid and 100 mg/l naphthalene acetic acid was applied to control boll shedding. 3:1 ratio was not observed in above said combinations. Some plants were found resistant against CLCuV by using petiole grafting technique. But no resistance was observed. Maximum tolerance was found in this combination, that is, [{.2 (*hirs.* x *G. anom.*) x ³*G. hirs.*} x {²*G. hirs.* x 2 (*G. arbo.* x *G. anom*) x ²*G. hirs.*}] x ²*G. hirs.* By using this material CIM-608 has been evolved which is having high tolerance to CLCuV; this will increase cotton production and will be a source of food security.

Key words: Cotton, introgression, cotton leaf curl virus (CLCuV), tolerant.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is the backbone of Pakistan's economy accounts for 8.6% of the value

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added in agriculture and about 1.8% to GDP. Total area for cotton cultivation was 3.106 million hectares 10.1% more than last year 2.82 million hectares. Estimated production is 12.7 million bales for 2009-2010, higher by 7.4% over last year production of 11.8 million bales. Yield was 695 kg/ha. However cotton production was 5.0% less than the target of 13.36 million bales mainly due to spread of Cotton Leaf Curl Virus (CLCuV), shortage of irrigation water, high temperature in August resulting high fruit shedding and flare up of sucking pests (Agricultural Statistics of Pakistan, 2009-2010). Cotton is infected by several insects, pests and pathogens inducing different diseases. Among them Cotton Leaf Curl Virus is the most obnoxious disease causing enormous losses to the cotton crop (Khan and Ahmad, 2005). It has caused reduction of 9.45 million bales during the last decade, amounting to rupees 75 billion to the national economy. In 1981, the cotton area affected by this disease was about 35000 acres (Ali et al., 1992). This disease spread in Sindh in 1997, in Khyber Pakhtoon Khawa in 1998 and in Baluchistan in 2001 (Tariq, 2005). Reduction in yield in tolerant varieties was 50% and 85 to 90% in susceptible cultivars (Hussain, 1995; Khan et al., 2001).

A new and mutant strain of CLCuV (Begomovirus) was observed in Burewala region 2001-2002, which is more virulent and named the Burewala Strain of Cotton Virus (BSCV) after being detected in Burewala. In the wake of resurgence of new strain of Burewala virus the resistance was broken down and as a result all the varieties including LRA-5166, CP-15/2 and Cedex (resistant to previous CLCuV became susceptible to BSCV (Bridson, 2003; Mahmood et al., 2003; Tahir et al., 2004).

Different scientists worked on interspecific hybridization for transferring resistant genes for favorable traits from wild diploid species into tetraploid cultivated cotton like (Blank and Leathers, 1963) transferred resistant genes against cotton rust caused by *Puccinia cacabata*. from *Gossypium anomalum* L. and *Gossypium arboreum* L. into *Gossypium hirsutum* L. through interspecific hybridization, induction of polyploidy and back crossing accompanied by continuous screening for resistance. It is worthwhile to combine the genes for Cotton Leaf Curl Virus resistance and other diseases and drought resistance between *G. hirsutum* L. and *G. arboreum* L. cotton (Amin, 1940). Moreover other research workers have attained success in introgression of *G. hirsutum* L. and *G. arboreum* L. (Bao-Liang et al., 2003). Moreover other research workers have attained success in interspecific introgression of *G. hirsutum* L. and *G. arboreum* L. (Bao-Liang et al., 2003). Similarly, introgressed resistant genes B6 found in 'A' genome of *G. arboreum* L. against bacterial blight caused by *Xanthomonas malvacearum*. Into *Gossypium barbadense* L (Knight, 1957; Brinkerhoff, 1970). Breeders also achieved most resistant commercial variety 'Auburn 56' against Root knot nematode (*Meloidogyne incognita*) in U. S. cotton through transgressive segregation

(Shepherd, 1974). Sacks and Robinson (2009) also introgressed resistance to *Rotylenchulus reniformis* into the tetraploid 2 (AD1) through crossing a resistant diploid A2-genome *G. arboreum* L. accession (A2-190) with a hexaploid 2 [(AD1) D4] bridging line (G 371) to obtain a tetraploid triple specie hybrid. At present no single variety of *G. hirsutum* L. is resistant to BSCV, whereas *G. arboreum* L. is known to have immunity against Cotton Leaf Curl Virus (Bird, 1973). Keeping in view losses made by CLCuV emphasis should be given to explore the possibility of successful transferring virus resistant genes from Desi cotton (*Gossypium arboreum* L. 2n=26) into cultivated upland cotton (*G. hirsutum* L. 2n = 52) genotypes through conventional breeding.

The Cytogenetics Section of Central Cotton Research Institute, Multan is engaged for the past many years in transferring desirable characters of wild species to the cultivated ones through complex crosses. The objective of this study is to introgress CLCuV resistance from wild species to upland cotton which will ultimately leads to the development of cotton leaf curl virus tolerant varieties. This will be a source of food security all over the world. In screening of available 30 *Gossypium* species to cotton leaf curl virus observed in field, it was found that the diploid species of cotton viz. *G. herbaceum*, *G. arboreum*, *G. anomalum*, *G. captis viridis*, *G. gossypoides*, *G. laxum*, *G. stocksii*, *G. areysianum*, *G. somalense* and *G. longicalyx* showed resistance to Burewala stain of cotton leaf curl virus.

MATERIALS AND METHODS

G. arboreum and *G. anomalum* were found resistant rather immune to cotton leaf curl virus. So *G. arboreum* and *G. anomalum* (diploids) were crossed as male with *G. hirsutum* (tetraploid) to transfer the virus resistance from both the diploid species into *G. hirsutum* background. Methods for developing virus resistant material are as follows:

1. *G. anomalum* Wawra et. Payer(2n= 26)-B1 was crossed with *G. hirsutum* Linn(2n=52)(AD)1 as female. The resultant triploid hybrid was treated with 0.2% aqueous solution of colchicine for 72 h using seedling dip method for doubling the chromosomes. The hexaploid was further crossed with *G. hirsutum* to make a pentaploid which was further back crossed four times to get a stable tetraploid.
2. *G. anomalum* Wawra et. Payer(2n= 26)-B1 was crossed with *G. arboreum* Linn(2n=26)A2. The resultant diploid inter-specific hybrid was treated with 0.2% aqueous solution of colchicine for 72 h using seedling dip method for doubling the chromosome. The resultant tetraploid hybrid was crossed and back crossed with *G. hirsutum*.
3. Both the above mentioned species hybrids viz., [$\{^4\text{hirs.} \times 2 (\text{hirs.} \times \text{G. anom.})\} \times \{^2\text{hirs.} \times 2 (\text{arbo.} \times \text{anom.})\}] \times \text{hirs.}$ were also crossed with each other.

The synthesized material was grafted with virus affected petioles of CIM-473 to check its virus resistance against BSCV in green house. Later on these resistant plants were shifted to field for assessment of their resistance against BSCV in field conditions. The number of the plants showing resistance in green house as well as in field was recorded.

The plants showing CLCuV resistance were selected and further

Table 1. Screening of resistant material during 2006-2007.

| Material | Total number of plants grafted in green house | Number of plants not showing symptoms and transplanted in the field | BSCV affected plants in the field | Resistant plant | Percentage resistance |
|---|---|---|-----------------------------------|-----------------|-----------------------|
| ⁴ <i>G. hirs.</i> x 2 (<i>hirs.</i> x <i>G. anom.</i>). | 303 | 13 | 11 | 2 | 0.66 |
| ² <i>G. hirs.</i> x 2(<i>G.arbo.</i> x <i>.anom.</i>) | 774 | 115 | 97 | 18 | 2.32 |
| [[⁴ <i>G. hirs.</i> x 2 (<i>G.hirs.</i> x <i>G. anom.</i>)] x { ² <i>G. hirs.</i> x 2(<i>G.arbo.</i> x <i>G.anom</i>)}] x <i>G. hirs</i> | 1354 | 214 | 173 | 41 | 3.0 |
| Total | 2431 | 342 | 281 | 61 | |

Table 2. Screening of material in the field during 2006-2007.

| Material | Total number of plants in the field | BSCV affected plants in the field | Resistant plant | Percentage resistance |
|--|-------------------------------------|-----------------------------------|-----------------|-----------------------|
| ⁴ <i>G. hirs.</i> x 2 (<i>G.hirs.</i> x <i>G. anom.</i>). | 1231 | 1193 | 38 | 3.1 |
| ² <i>G. hirs.</i> x 2(<i>G.arbo.</i> x <i>G.anom</i>) | 121 | 85 | 36 | 29.7 |
| [[⁴ <i>G. hirs.</i> x 2 (<i>hirs.</i> x <i>G. anom.</i>)] x { ² <i>G. hirs.</i> x 2(<i>G.arbo.</i> x <i>G.anom</i>)}] x <i>G. hirs.</i> | 88 | 59 | 29 | 33.0 |
| Total | 1440 | 1337 | 103 | |

selection was done by using progeny row trials. Number of strains have been developed which are having good CLCuV tolerance.

RESULTS

By conventional breeding methods, crosses between the two species of cotton are rarely successful due to abortion of embryo after fertilization. The diploid species that cross directly with upland cotton produce sterile triploid F₁ hybrids. Such triploid hybrids have to be treated with Colchicine to produce hexaploids (Joshi and Johri, 1972). We synthesized Triploid hybrid plants of (*G. hirs.* L. x *G. anom.*) L. and 2 (*G. arbo.* x *G. anom*) treated with 0.1% Colchicine solution for seven days using cotton swab method. There was no effect of Colchicine as these plants were old.

2431 plants of the material developed by species hybrids were grafted with virus affected of petiole of CIM-473 to check their virus resistance against BSCV in green house. Out of these 342 plants which did not show BSCV symptoms were transplanted in the field. Only 61 plants showed resistant against BSCV till maturity of crop. The results are given in Table 1. The table shows that in the first *Gossypium* species hybrid viz., ⁴*G. hirs* x 2(*G. hirs* x *G. anom*), resistance to BSCV was 0.66%, where *G. anomalum* alone resistant to BSCV was used. While in the second combination viz., ²*G. hirs* x 2(*G. arbo* x *G. anom*), the resistance to BSCV was 2.32%. When two *Gossypium*, that is, *G. arboreum*. and *G. anomalum* resistant to BSCV were used. In the third combination,

that is, [[⁴*G. hirs.* x 2 (*hirs.* x *G. anom.*)] x {²*G. hirs.* x 2(*G.arbo.* x *G.anom*)}] x *G. hirs.* where *G. anomalum* has been used twice and *G. arboreum* once, the resistance against BSCV was 3.0%. Our results are also in agreement with those of (Altman, 1988) that in the development of F₁ hybrids and BC₁'s plants application of exogenous hormone techniques are superior to *in vitro* methods.

The neutral plants neither grafted in greenhouse nor in the field were tested against BSCV. Data given in the Table 2 is also in agreement with that of Table 1. The resistance to BSCV in the first, second hybrid, and in their combination was 3.1, 29.7 and 33.0% respectively. The plants which showed resistance against cotton leaf curl virus were picked, ginned and their fiber quality was evaluated.

The economic and fibre characters of some of the resistant material are given in Tables 3 to 6. Virus resistant plants in the interspecific hybrid ⁴*G. hirs.* x 2 (*G. arbo.* x *G. anom.*) having very good lint percentage were CP-38/Z55 (45.9), Z52A, CP-37/Z50 (44.2) and Z52 (42.2); while virus resistant plants have extra long fiber length were Z-69 (31.3 mm), CP-38/Z55 (28.3 mm), Z52A (30.5 mm), CP-37/Z50 (30.4 mm) and Z52 (31.0 mm) (Table 4). Neutral plants of interspecific hybrid ⁴*G. hirs.* x 2 (*G. arbo.* x *G. anom.*) having better lint percentage and fiber length as compared to standard were CP-30 (39.5%, 31.5 mm), CP-31 (46.6%, 28.6 mm), CP-32 (38.6%, 31.6 mm), CP-36 (45.4%, 29.0 mm), CP-37 (45.0%, 29.0 mm) and CP-38 (46.3%, 26.5 mm) as it is shown in Table 4.

Table 3. Performance of resistant plants of ⁴G *hirs.* × 2 (*G. hir.* × *G. anom.*)

| Hybrid No. | Seed cotton yield (g) | Lint (%) | Fibre length (mm) | Fibre fineness (µg/inch) | Fibre strength g/tex |
|--|-----------------------|----------|-------------------|--------------------------|----------------------|
| ⁴ G <i>hirs.</i> × 2 (<i>G. hir.</i> × <i>G. anom.</i>) | | | | | |
| CP13/Z38 | 55.0 | 38.9 | 26.1 | 5.0 | 28.2 |
| Z40 | 204.3 | 40.2 | 29.8 | 4.5 | 27.3 |
| ³ G <i>hirs.</i> × 2 (<i>G. hir.</i> × <i>G. anom.</i>) | | | | | |
| CP13 (2004) | 287.4 | 37.4 | 26.9 | 4.6 | 23.7 |
| ² G <i>hirs.</i> × 2 (<i>G. hir.</i> × <i>G. anom.</i>) | | | | | |
| P6 (2003) | 37.6 | 36.9 | 26.2 | 4.4 | 27.1 |

Table 4. Performance of resistant plants of *G. hirs.* × 2 (*G. arbo.* × *G. anom.*).

| Plant No. | Seed cotton Yield (g) | Lint (%) | Fibre length (mm) | Fibre fineness (µg/inch) | Fibre strength (g/tex) |
|--|-----------------------|----------|-------------------|--------------------------|------------------------|
| Resistant plants of ⁴G. <i>hirs.</i> × 2 (<i>G. arbo.</i> × <i>G. anom.</i>) | | | | | |
| CP-37/Z50 | 117.4 | 42.2 | 30.4 | 3.3 | 36.1 |
| Z51 | 47.4 | 41.9 | 29.8 | 3.2 | 33.7 |
| Z52 | 117.8 | 42.2 | 31.0 | 3.9 | 34.5 |
| Z52A | 25.06 | 44.0 | 30.5 | 4.0 | 31.1 |
| Z54 | 122.2 | 42.0 | 28.2 | 5.3 | 27.8 |
| CP-38/Z55 | 47.6 | 45.9 | 28.3 | 2.9 | 36.4 |
| Z56 | 87.0 | 36.3 | 29.9 | 4.3 | 30.1 |
| AB1/Z61 | 57.2 | 39.9 | 29.7 | 3.3 | 31.8 |
| Z67 | 41.0 | 34.1 | 33.3 | 3.5 | 34.6 |
| Z69 | 218.4 | 39.5 | 31.3 | 3.4 | 35.5 |
| Neutral plants of ⁴G. <i>hirs.</i> × 2 (<i>G. arbo.</i> × <i>G. anom.</i>) | | | | | |
| CP-29 | 184.3 | 43.2 | 28.6 | 5.0 | 26.0 |
| | 96.0 | 41.5 | 28.4 | 4.4 | 31.2 |
| | 225.5 | 43.9 | 28.2 | 5.8 | 24.9 |
| CP-30 | 166.6 | 34.6 | 31.8 | 3.5 | 35.3 |
| | 112.2 | 38.3 | 30.7 | 4.2 | 34.6 |
| | 30.4 | 39.5 | 31.5 | 4.3 | 32.8 |
| CP-31 | 75.3 | 43.6 | 28.1 | 3.8 | 28.7 |
| | 33.0 | 46.6 | 28.8 | 4.1 | 29.6 |
| | 58.4 | 41.6 | 30.2 | 3.6 | 32.2 |
| CP-32 | 292.0 | 38.8 | 31.6 | 3.3 | 33.0 |
| CP-36 | 108.2 | 45.4 | 29.0 | 3.0 | 37.8 |
| CP-37 | 230.0 | 45.0 | 29. | 3.8 | 31.6 |
| | 71.0 | 43.7 | 31.0 | 3.2 | 32.8 |
| CP-38 | 62.0 | 46.3 | 26.5 | 4.8 | 25.6 |
| AB1 | 74.7 | 41.8 | 28.8 | 3.1 | 32.6 |
| | 74.2 | 39.6 | 27.8 | 3.4 | 30.9 |
| | 64.3 | 40.0 | 29.4 | 3.4 | 30.8 |
| P-22 (2004) | 24.0 | 33.3 | 31.1 | 3.2 | 32.8 |
| CIM-496 (C.S.) | 90.3 | 41.2 | 28.9 | 4.8 | 26.5 |
| CIM-506 (C.S.) | 86.1 | 37.2 | 28.5 | 4.7 | 26.3 |

Table 5. Performance of of resistant plants of [$\{^3G. hirs. \times 2 (G. hir. \times G. anom.)\} \times \{^2G. hirs. \times 2(arbo. \times G. anom.)\} \times G. hir.$]

| Plant No. | Seed cotton yield/plant (g) | Lint (%) | Fibre length (mm) | Fibre fineness ($\mu\text{g}/\text{inch}$) | Fibre strength (g/tex) |
|---------------|-----------------------------|----------|-------------------|--|------------------------|
| CP-3/Z2 | 23 | 44.1 | 28.9 | 3.6 | 32.2 |
| CP-4/Z3 | 254 | 38.4 | 29.4 | 3.9 | 29.1 |
| Z4 | 114 | 37.7 | 33.4 | 3.5 | 32.5 |
| Z5 | 418 | 41.9 | 32.3 | 4.1 | 29.1 |
| Z6 | 303 | 39.0 | 34.3 | 3.4 | 31.9 |
| Z8 | 116.6 | 36.7 | 32.0 | 3.1 | 32.5 |
| CP-5/Z15 | 60.3 | 35.2 | 33.8 | 3.6 | 29.9 |
| Z16 | 76.3 | 35.9 | 31.8 | 3.5 | 29.9 |
| Z18 | 141.0 | 35.1 | 30.0 | 4.1 | 29.1 |
| CP-7/Z24 | 86.0 | 38.6 | 30.4 | 4.5 | 33.9 |
| Z27 | 148.2 | 35.4 | 30.9 | 4.4 | 30.2 |
| Z28 | 153.4 | 45.6 | 28.3 | 3.8 | 30.3 |
| Z29 | 26.4 | 41.7 | 27.8 | 3.9 | 31.6 |
| CP-8 | 188.8 | 39.4 | 29.0 | 4.8 | 27.0 |
| CP-11/Z32 | 120.4 | 45.3 | 31.1 | 3.4 | 36.0 |
| Z34 | 144.3 | 37.6 | 32.3 | 3.6 | 34.2 |
| Z42A | 89.0 | 40.1 | 30.0 | 3.3 | 34.5 |
| CP-12/Z36 | 234.6 | 35.4 | 30.0 | 4.5 | 28.4 |
| Z37 | 153.0 | 39.2 | 30.3 | 3.3 | 33.4 |
| CP-17/H3 | 14902 | 38.6 | 29.1 | 4.3 | 28.7 |
| H7 | 83.8 | 37.8 | 29.3 | 4.1 | 28.7 |
| H12 | 200.3 | 44.4 | 28.1 | 3.7 | 29.2 |
| H13 | 205.0 | 36.0 | 29.4 | 4.4 | 30.8 |
| H14 | 353.5 | 39.5 | 30.9 | 4.0 | 29.8 |
| H15 | 64.2 | 41.1 | 28.5 | 3.7 | 29.5 |
| H16 | 130.0 | 35.4 | 30.8 | 4.0 | 30.0 |
| CP-17 | 293.5 | 39.8 | 30.7 | 3.7 | 30.3 |
| CP-24/Z47 | 261.8 | 35.0 | 31.7 | 3.2 | 32.2 |
| P6 (2003) | 37.6 | 36.9 | 26.2 | 4.4 | 27.1 |
| P-22 (2004) | 24.0 | 33.3 | 31.1 | 3.2 | 32.8 |
| CIM-496 (C.S) | 90.3 | 40.2 | 28.1 | 4.8 | 26.5 |
| CIM506 (C.S.) | 86.1 | 37.2 | 27.8 | 4.9 | 26.3 |

Data in the Table 5 showed that good combinations of economic and fibre characteristics are available in this material synthesized through multiple species hybrids. Virus resistant plants in the multiple species interspecific hybrid [$\{^3G. hirs. \times 2 (G. hir. \times G. anom.)\} \times \{^2G. hirs. \times 2(arbo. \times G. anom.)\} \times G. hir.$] having very good lint percentage were CP-7/Z28 (45.6%), CP-11/Z32 (45.3%), CP-17/H12 (44.4%) and CP-3/Z2 (44.1%) while virus resistant plants exhibiting extra long fiber length were CP-3/Z6 (34.3 mm), CP-5/Z15 (33.8 mm), CP-4/Z5 and CP-11/Z34 (32.3 mm), CP-5/Z16 (31.8 mm) and CP-11/Z32 (31.1 mm). Virus resistant plants having good seed cotton yield with good fiber quality traits will be utilized in breeding programme for introgression of cotton leaf curl virus resistance in elite interspecific

combinations. The material, synthesized above, showed progressive increase in yield and ginning out turn percentage with improved fibre qualities.

Data in Table 6 revealed that outstanding combinations of different economic and fibre characteristics are present in this material. The cotton leaf curl virus resistant plants were selfed and seeds of this material were sown in the field in plant to progeny row to find out homozygous plants for virus resistance.

Elite strains developed by using interspecific combinations have very good tolerance to cotton leaf curl virus and having desirable fiber traits (Table 7). CIM-608 is the first introgressed strain has been submitted for approval to expert sub-committee in 2012 while Cyto-124 is showing high tolerance to cotton leaf curl virus with

Table 6. Performance of of resistant plants (Neutral plants).

| Plant No. | Seed cotton yield/plant (g) | Lint (%) | Fibre length (mm) | Fibre fineness ($\mu\text{g}/\text{inch}$) | Fibre strength (g/tex) |
|-----------|-----------------------------|----------|-------------------|--|------------------------|
| CP-5 | 61.5 | 35.8 | 31.7 | 3.1 | 37.6 |
| CP-6 | 166.3 | 37.3 | 29.4 | 3.9 | 34.6 |
| CP-11 | 129.0 | 38.8 | 30.4 | 3.5 | 34.7 |
| CP-17 | 336.5 | 35.5 | 30.6 | 4.1 | 29.9 |
| | 80.6 | 39.7 | 30.3 | 3.9 | 31.4 |
| | 141.1 | 38.6 | 30.1 | 4.6 | 30.0 |
| | 131.0 | 38.9 | 29.9 | 4.1 | 31.0 |
| | 97.8 | 40.2 | 29.4 | 4.2 | 28.9 |
| | 207.8 | 37.5 | 30.7 | 4.1 | 31.4 |
| CP-21 | 59.5 | 44.9 | 32.2 | 2.8 | 32.6 |
| CP-24 | 100.8 | 43.3 | 29.5 | 3.2 | 32.7 |
| | 103.0 | 40.8 | 30.9 | 3.6 | 35.0 |
| CP-25 | 121.3 | 50.5 | 27.5 | 4.1 | 29.9 |

Table 7. Elite strains developed through interspecific hybridization.

| Strains No. | Virus (%) | Lint (%) | Fibre length (mm) | Fibre fineness ($\mu\text{g}/\text{inch}$) | Fibre strength (tppsi) |
|-------------|-----------|----------|-------------------|--|------------------------|
| CIM-608 | 24.9 | 39.9 | 28.3 | 4.8 | 93.5 |
| Cyto-120 | 3.6 | 39.6 | 29.7 | 4.5 | 99.9 |
| Cyto-110 | 1.4 | 39.2 | 31.3 | 4.2 | 96.7 |
| Cyto-111 | 3.8 | 42.5 | 29.3 | 4.9 | 99.5 |
| Cyto-112 | 4.2 | 42.6 | 28.8 | 4.8 | 100.6 |
| Cyto-113 | 7.6 | 41.9 | 28.8 | 4.9 | 97.2 |
| Cyto-114 | 11.9 | 40.4 | 29.1 | 4.8 | 97.5 |
| Cyto-115 | 6.4 | 44.2 | 28.2 | 4.8 | 100.5 |
| Cyto-116 | 9.2 | 42.9 | 29.2 | 4.6 | 101.5 |
| Cyto-117 | 4.7 | 41.1 | 30.5 | 4.4 | 103.4 |
| Cyto-118 | 5.2 | 41.2 | 30.3 | 4.4 | 102.3 |
| Cyto-119 | 8.2 | 43.1 | 29.2 | 4.8 | 96.6 |
| Cyto-122 | 13.4 | 39.8 | 29.1 | 4.4 | 92.6 |
| Cyto-124 | 2.7 | 40.8 | 29.2 | 4.8 | 99.6 |

very good fiber traits.

DISCUSSION

G. hirsutum L. has low genetic diversity and lack of resistance against Cotton Leaf Curl Virus while wild diploid species of *Gossypium* have potential for resistance against many disasters like insect, pests, diseases and many abiotic factors. Hence there is great need for exploitation of this source for developing resistance against CLCV in cultivated tetraploid species. The primary factor limiting the success of interspecific hybridization in cotton is lack of retention of crossed bolls. Cotton breeders have been trying to obtain hybrids between diploid and tetraploid species for a long time

(Gill and Bajaj, 1987) but it has been difficult and sometimes impossible to obtain hybrids under *in situ* conditions because of several incompatibility factors. Weaver (1957) found ovule failure in the cross, *G. hirsutum* L. \times *G. arboreum* L. due to deranging effect of the hybrid embryos upon endosperm development, while in reciprocal cross (Weaver, 1958) abnormal endosperm nuclei mitosis and improper embryo differentiation were the main causes. Many workers used ovule and embryo culture for *Gossypium* interspecific hybridization (Stewart and Hsu, 1978; Refaat et al., 1984; Gill and Bajaj, 1987; Umbeck and Stewart, 1985; Thengane et al., 1986; 1987; Mirza et al., 1993) but Louant et al., (1977) was of the opinion that hybrids obtained by those workers could be obtained through standard hybridization. Interspecific hybridization of cotton has been assisted by exogenous

hormone application after pollination. Numerous reports from China have documented that exogenous hormone application alone can overcome certain crossing barriers within *Gossypium* (Liang and Sun, 1982; Liang et al., 1978). Gibberellic acid was used as growth regulator to obtain interspecific hybrids between tetraploid *G. hirsutum* L. and diploid *G. arboreum* L. species of cotton (Mofidabadi, 2009).

The plant hormones are known to control pollen tube growth (Kovaleva et al., 2005). Exogenous application of growth hormone has been used to facilitate interspecific crosses in many crops, that is, cotton (Altman, 1988), wheat (Sitch and Snap, 1987) and tomato (Gordillo et al., 2003). Altman (1988) compared exogenous application with *in vitro* techniques, that is, ovule and embryo culture and found that exogenous hormones used with standard hybridization were superior to *in vitro* methods.

Conclusion

Cotton leaf Curl Virus is menace for cotton production in the world and severally damaged the cotton crop in Pakistan. As there is no resistant genotype in upland cotton to CLCuV, the only way is to introgress this resistance in upland cotton from wild species. By using resistant wild species hybrids the material having very good economic traits with high seed cotton yield has been developed. CIM-608 has been approved for general cultivation in 2013 which will increase seed cotton production and will be a source of food security; Cyto-124 has shown lowest virus for the last two years in national coordinated varietal trial and has been submitted for approval in expert sub-committee. Moreover; this material will be used for insect resistance having good fiber quality.

Conflict of Interest

The authors have not declared any conflict of interest.

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