Evaluation of Cotton Leaf Curl Virus Resistance in BC$_1$, BC$_2$, and BC$_3$ Progenies from an Interspecific Cross between *Gossypium arboreum* and *Gossypium hirsutum*

Wajad Nazeer$^{1,2}$, Abdul Latif Tipu$^2$, Saghir Ahmad$^2$, Khalid Mahmood$^2$, Abid Mahmood$^3$, Baoliang Zhou$^1$*

$1$ State Key Laboratory of Crop Genetics and Germplasm Enhancement, MOE Hybrid Cotton R&D Engineering Research Center, Nanjing Agricultural University, Nanjing, Jiangsu Province, China, $2$ Cotton Research Station, Multan, Ayub Agricultural Research Institute, Faisalabad, Punjab, Pakistan, $3$ Cotton Research Institute, Ayub Agricultural Research Institute, Faisalabad, Punjab, Pakistan

**Abstract**

Cotton leaf curl virus disease (CLCuD) is an important constraint to cotton production. The resistance of *G. arboreum* to this devastating disease is well documented. In the present investigation, we explored the possibility of transferring genes for resistance to CLCuD from *G. arboreum* (2n = 26) cv 15-Mollisoni into *G. hirsutum* (2n = 52) cv CRSM-38 through conventional breeding. We investigated the cytology of the BC$_1$ to BC$_3$ progenies of direct and reciprocal crosses of *G. arboreum* and *G. hirsutum* and evaluated their resistance to CLCuD. The F$_1$ progenies were completely resistant to this disease, while a decrease in resistance was observed in all backcross generations. As backcrossing progressed, the disease incidence increased in BC$_1$ (1.7–2.0%), BC$_2$ (1.8–4.0%), and BC$_3$ (4.2–7.0%). However, the disease incidence was much lower than that of the check variety CIM-496, with a CLCuD incidence of 96%. Additionally, the disease incidence percentage was lower in the direct cross (2(G. arboreum) × G. hirsutum) than in that of G. hirsutum × G. arboreum. Phenotypic resemblance of BC$_1$ to BC$_3$ progenies to *G. arboreum* confirmed the success of cross between the two species. Cytological studies of CLCuD-resistant plants revealed that the frequency of univalents and multivalents was high in BC$_1$, with sterile or partially fertile plants, but low in BC$_2$ (in both combinations), with shy bearing plants. In BC$_3$, most of the plants exhibited normal bearing ability due to the high frequency of chromosome associations (bivalents). The assessment of CLCuD through grafting showed that the BC$_1$ to BC$_3$ progenies were highly resistant to this disease. Thus, this study successfully demonstrates the possibility of introgressing CLCuD resistance genes from *G. arboreum* to *G. hirsutum*.

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* Email: baoliangzhou@njau.edu.cn

**Introduction**

Cotton production is biotically constrained by various diseases, which lead to yield instability and reduced seed quality. Cotton leaf curl disease (CLCuD) is a debilitating disease of cotton in Africa, Pakistan, and Northwestern India [1–3]. CLCuD is caused by a pathogen complex of a virus and a DNA beta satellite (DNA-β) molecule [4]. There are seven such virus species, all belonging to the *Begomovirus* genus, and DNA-β satellites are associated with CLCuD in these regions [3–8].

CLCuD was first recorded in 1967 in the Multan district, Pakistan, on scattered *Gossypium hirsutum* plants [9–11], and it has spread rapidly to all cotton growing areas of Pakistan and throughout the Indian subcontinent. Two epidemics of this disease have been observed during the past three decades due to a loss of host-plant resistance in existing cotton varieties [12–13].

In Pakistan, an outbreak of CLCuD occurred in the early 1990s. This disease devastated the Pakistani cotton industry, where it caused an estimated yield reduction of 30–35%. Between 1992 and 1997, the economic losses due to CLCuD in Pakistan amounted to approximately 5 billion dollars (US) [14]. Similarly, in the Indian state of Punjab, this disease reduced cotton production by almost 70% in 1998 [15]. Singh et al. [16] observed a reduction of 52.7% in the number of bolls and a reduction of 54.2% in boll weight due to CLCuD, whereas the differences in yield loss between resistant and susceptible cultivars were almost 50% and 85–90%, respectively.

In the late 1990s, several resistant cotton varieties were gradually introduced into the Indo-Pak region, and losses due to the disease diminished [17–18]. However, resistance subsequently broke in 2001–2002 [3,12] due to new strains of CLCuD emerged, and all of the cotton varieties that were previously known resistant to CLCuD, such as LRA-5166, CP-15/2, and Cedex, have...
become susceptible to CLCuD [6–7,19–23]. Symptoms of this disease were also reported in China [24], which is located far from the hot spots of India and Pakistan, and there is great concern that CLCuD could spread from its origin to other cotton growing areas of the world where the disease is not currently present. Plant biologists have attempted to understand the molecular biology of this disease complex to control CLCuD [25], but the tricky nature of the pathogen and the rapid evolution/recombination of these genes have hindered the progress of this research [26–28].

In plant breeding, wild relatives have long been studied due to the presence of novel genes [29–31], and these wild species have been exploited most often as sources for biotic and abiotic stress resistance [32]. Among the wild species of cotton, especially, desi cotton (G. arboreum L.) has built in desirable resistant genes for all kind of Begomoviruses associated with CLCuD [33]. Additionally, G. arboreum is known to combat various stresses like drought [34–35], heat [36], root rot, cotton leaf curl virus [37] and insect pests (bollworms and aphids) [12]. Interspecific hybridization of cotton has been performed with varying degrees of success [21,38–40]. For example, Sacks and Robinson [41] transferred nematode (Rotylenchulus reniformis) resistance into tetraploid G. hirsutum. Chen et al. [42] and Nazeer et al. [2] employed Gossypium australis and Gossypium stocksii to introgress some novel genes for drought and CLCuD resistance into G. hirsutum, respectively. The interspecific hybridization is quite difficult, especially, between G. arboreum and G. hirsutum, and some scientists explored bridge lines for introgression of genes form wild species [43].

At present, no single variety of G. hirsutum is resistant to CLCuD; however, G. arboreum is documented to have resistance against CLCuD [31]. Due to the importance of this disease and significant features of this species, we initiated a project to explore the possibility of successful transferring CLCuD resistance genes from Desi cotton (G. arboreum, 2n = 26) into cultivated upland cotton (G. hirsutum, 2n = 52) genotypes through conventional hybridization and backcrossing without developing bridging line. In this way maximum desirable donor genes of G. arboreum can be transferred into G. hirsutum to improve the resistance to CLCuD of the cultivated G. hirsutum.

Materials and Methods

Plant materials

The plant materials used in this study include G. hirsutum cv CRSM-38 (2n = 4x = AADD = 52), G. arboreum cv 15-Mollisoni (2n = 2x = AA = 26), and an artificial autotetraploid of G. arboreum cv 15-Mollisoni (2n = 4x = 52; Figure 1). The F1 CLCuD-resistant progeny involving these parents, which was developed by Ahmad et al. [44], comprising direct cross [2(G. arboreum)×G. hirsutum] and its reciprocal cross (G. hirsutum ×G. arboreum), was utilized to directly backcross with G. hirsutum. The number of F1 progenies was increased by cuttings. Thus total number of F1 plant progenies for direct and reciprocal cross was 10 and 15, respectively, to generate BC1 to BC2 generations. CIM-496, a cotton variety highly susceptible to CLCuD, was employed as a standard/control in order to obtain a natural virus inoculum.

Development of backcross progenies

The F1 CLCuD-resistant progenies consisting of two cross combinations, 2(G. arboreum)×G. hirsutum and G. hirsutum ×G. arboreum, were backcrossed with G. hirsutum to produce the BC1 progenies in 2011. The BC1 progenies were planted in the field at Cotton Research Station in Multan, Pakistan in May 2012 and backcrossed with G. hirsutum to generate the BC2 progenies. These BC2 progenies were again backcrossed with G. hirsutum cv CRSM-38 to produce the BC3 progenies in 2013. One thing should be noticed here that only those normal morphological plant progenies that produce more fruits but no symptoms of CLCuD were selected for backcrossing. The plant progenies that showed even minor spots of CLCuD were rejected to utilize for backcrossing. The scheme for the development of the backcross progenies are shown in Figure 2. Emasculation was carried out in the evening, and emasculated flowers were manually pollinated the next morning.

Use of plant growth hormones for hybridization

Normally, embryos fail to develop in hybridizations between G. arboreum and G. hirsutum. This obstacle was overcome by the application of plant hormones such as gibberellic acid (GA3) and naphthalene acetic acid. Specifically, 50 mgL−1 GA3 and 100 mg L−1 naphthalene acetic acid were applied to the bases of pedicles 24 hours after pollination for 3 consecutive days to reduce embryo and boll shedding. The number of cross boll sets was counted, and the bolls were picked at harvest time.

Cross fertility studies

Fertility studies for BC1 to BC3 progenies of 2(G. arboreum)×G. hirsutum and G. hirsutum ×G. arboreum were measured in term of cross boll setting and their germination percentage by given formula:

Boll setting(%) = Total number of cross boll picked Total number of pollinations × 100;

Seed germination(%) = Number of seed gminated Total number of seed tested × 100;

Figure 1. Parents of interspecific hybridization. A. G. arboreum cv 15-Mollisoni (2n = 2x = 26); B. G. hirsutum cv CRSM-38 (2n = 4x = 52); C. 2(G. arboreum) (2n = 4x = 52); D. [2(G. arboreum)×G. hirsutum] F1 (2n = 4x = 52); E. (G. hirsutum ×G. arboreum) F1 (2n = 3x = 39).

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Morphological characteristics

Observations of growth habit, stem color, leaf shape, leaf hairiness, bracteole size, corolla color, petal spots, the position of the staminal column, anther color, and dehiscence in the parents, as well as in BC1 to BC3, were recorded. The phenotypic resemblance of BC1 to BC3 progenies to G. arboreum having desirable traits with good resistance to CLCuD will be helpful for selection of introgression progenies.

Cytological studies

Morphological normal plants producing more fruits were selected from BC1 to BC3 progenies for cytological studies. Young buds of BC1 to BC3 plants, along with those of the parents, were collected and fixed in Carnoy’s solution at 8 to 9 am and preserved in 70% ethanol after 24 h. Three to four anthers were squashed on a slide with a drop of 2.5% acetocarmine solution to examine the pollen mother cells (PMCs). Chromosomal configurations such as univalent (I’s), bivalents (II’s), trivalents (III’s), quadrivalents (IV’s), and division stage were examined under a Labomed microscope, and photographs were also taken using a camera mounted on a Labomed microscope.

Maintenance of virus inoculum and screening for CLCuD

Artificial inoculation techniques is not available for CLCuD, therefore, the only way to study the response of cotton germplasm is to expose the introgression progenies to high inoculum pressure by planting in natural hot spots [45], so sick plot technique was used to arrange spreader plants among BC introgression lines. In this sick plot technique, we planted susceptible variety CIM-496 after each two rows of CLCuD resistant lines to encourage uniform spread of the disease. Planting of BC1 to BC3 progenies was done after 3rd week of May for the three seasons i.e. 2011–2013. Sowing was done manually and row to row (75 cm) and plant to plant (30 cm) distance was maintained. Row length for each genotype was 450 cm and plot size was variable depending upon the seed availability.

Phenotypic assessment of BC1 to BC3 progenies against CLCuD

The resistance of the BC1 to BC3 progenies against CLCuD was assessed under natural field conditions using an inoculum of CIM-496 at Cotton Research Station in Multan, Pakistan which is hot spot of CLCuD. Data for CLCuD were recorded following the rating system described in Table 1 to calculate the severity index.

| Disease index (%) | Severity grade | Symptoms | Remarks |
|-------------------|----------------|----------|---------|
| 0                 | 0              | No Symptoms | Resistant |
| 1–20              | 1              | Thickening of only secondary and tertiary veins. | Highly tolerant |
| 21–30             | 2              | Thickening of secondary and primary (mid rib) veins. | Tolerant |
| 31–50             | 3              | Vein thickening (V.T), leaf curling (L.C) or enation (E) or both. | Susceptible |
| >50               | 4              | Stunting along with vein thickening leaf curling/enation. | Highly susceptible |

Table 1. Disease rating (symptom rating) scale for evaluation of cotton leaf curl virus disease.
(SI), percent disease index (%), DI, and disease reaction. Individual plant ratings for each genotype were added and means were calculated to generate the corresponding SI. The DI was calculated using the following formula:

$$\text{Percent disease index} = \frac{\text{Sum of all disease ratings}}{\text{Total plants}} \times \frac{\text{100}}{\text{Maximum grade}}$$

The percent disease tolerance (PDT) was calculated by selecting a minimum of 100 plants on a diagonal from one corner to the other, and diseased plants were counted to determine the PDT using the formula:

$$\text{Percent disease tolerance} = \frac{\text{Total plants} - \text{diseased plants}}{\text{Total plants}} \times 100$$

Data regarding the latent period, number of virus-infected plants, disease incidence percentage, disease severity index, infection type, and disease reaction were recorded.

Inoculation of CLCuD through grafting

A petiole and rootstock from CIM-496 were used to transfer virus inoculum into healthy plants. Two grafting techniques, i.e., approach grafting and petiole grafting, were employed to confirm the resistance against CLCuD in BC1 to BC3 plants. For approach grafting, the resistant plants of the BC1, BC2, and BC3 progenies were used as scions, whereas virus-susceptible *G. hirsutum* plants were used as stock. For petiole grafting, young petioles from CLCuD-infected plants were selected and inserted into the test plants. Two infected petioles were also grafted onto the same plant to introduce additional virus inoculum. The following data were recorded: grafting success, infectivity, latent period, infection type, disease severity index, and disease incidence percentage at 40 and 70 days after grafting (DAG).

## Results

### Cross fertility studies

Examination of the cross ability of BC1 to BC3 of the combination 2(*G. arboreum*) × *G. hirsutum* and *G. hirsutum* × *G. arboreum* revealed that the maximum percentage of boll set (42.9%) and germination (67.0%) were observed in the BC3 (*G. hirsutum* × *G. arboreum*) progenies (Table 2). Minimum boll setting (1.3%) was recorded in the cross BC2 (*G. hirsutum* × *G. arboreum*). A minimum percentage of viable seeds (35.1%) were obtained in BC1 (*G. arboreum* × *G. hirsutum*). The boll setting and germination (%) gradually increased from BC1 to BC3 (Figure 3).

| Year     | No. of plants | No. of pollinations | No. of bolls picked | No. of seed obtained | No. of seed germinated | Boll setting (%) | No. of seed germinated | Germination (%) |
|----------|---------------|---------------------|---------------------|----------------------|------------------------|------------------|------------------------|----------------|
| BC1 [2(*G. arboreum*) × *G. hirsutum*) | 2009-11       | 15                  | 1,0890              | 266                  | 8144                   | 12               | 19                     | 2               |
| BC1 [(*G. hirsutum*) × *G. arboreum*) | 2009-11       | 12                  | 1,044               | 19                   | 1,051                  | 14               | 17                     | 5               |
| BC2 [2(*G. arboreum*) × *G. hirsutum*) | 2012          | 155                 | 1,495               | 51                   | 1,429                  | 2               | 80                     | 4               |
| BC2 [(*G. hirsutum*) × *G. arboreum*) | 2012          | 12                  | 299                 | 24                   | 723                    | 2               | 723.3                  | 67.0           |
| BC3 [2(*G. arboreum*) × *G. hirsutum*) | 2013          | 225                 | 980                 | 15                   | 980                    | 12               | 980                    | 42.9           |
| BC3 [(*G. hirsutum*) × *G. arboreum*) | 2013          | 225                 | 7,203               | 24                   | 7,203                  | 2               | 7,203                  | 67.0           |

*Number of plants used for pollination and recording data. doi:10.1371/journal.pone.0111861.t002

### Morphological studies

Examination of the morphological characteristics of the parents and BC1 to BC3 of 2(*G. arboreum*) × *G. hirsutum* revealed that in BC1 to BC3, leaf hairiness, flower size, corolla color, petal spots, and pollen color were segregated for the male and female parents. Stem color, leaf lobation, flower size, corolla color, petal number, petal size, anther dehiscence, and pollen color of BC1 to BC3 were similar to those of the female parents. Stem hairiness, gossypol glands, leaf size, leaf hairiness, leaf texture, petiole length, and bracteole number and size were dominant characters of the male parents. Bracteole dentation, petiole size, petal spots, and position

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Table 2. Fertility studies of interspecific hybrid between *G. arboreum* and *G. hirsutum* to produce the BC1 to BC3 progenies.
of the staminal column of BC1 to BC3 were intermediate between those of both parents (Table 3). The hybrid plant progenies of BC1 to BC3 of 2(*G. arboreum*) × *G. hirsutum* are shown in Figure 4.

An analysis of the morphological characteristics of the parents and BC1 to BC3 of *G. hirsutum × G. arboreum* revealed that in BC1 to BC3, gossypol, bracteole number and size, and pistil size was dominant characters of the female parents. Leaf size, leaf lobation, corolla color, petal spots, and pollen color were the dominant characteristics of the male parents. Stem color and hairiness, leaf texture, bracteole number and size, and position of staminal column of BC1 to BC3 were intermediate between those of both parents, while leaf hairiness was segregated (Table 4). The hybrid plant progenies of BC1 to BC3 of *G. hirsutum × G. arboreum* are shown in Figure 5.

Morphological characteristics particularly leaf texture, leaf size, bracteole size, corolla color and petal spots from *G. arboreum* into BC1 to BC3 progenies of both crosses helped for selection of plant progenies that have some resemblance of *G. arboreum* and also showed CLCuD resistance.

Cytological studies

**Meiosis in parents.** The course of meiosis was examined in the *G. hirsutum* and *G. arboreum* parents. In these species, the reduction division was normal, with regular pairing of chromosomes. The number of bivalents in *G. hirsutum* and *G. arboreum* at Metaphase-I was 26 and 13, respectively (Figures 6A and 6B). The disjunction of the chromosomes was normal at Anaphase-I.

The meiotic behavior in the artificial autotetraploid of *G. arboreum* parent showed two I’s, 23 II’s, and one IV (Figure 6C).

**Meiosis in BC1.** [2(*G. arboreum*) × *G. hirsutum*]. The progenies of this combination comprised 15 plants; only seven normal morphological plants with better boll setting were studied cytologically. Cytological studies at Metaphase-I revealed that there were six I’s, 21 II’s, and one IV’s (Figure 6D). The number of I’s, II’s, III’s, and IV’s for 76 PMCs ranged from 5–12, 18–22, 0–1, and 0–1, respectively, for a total of 52 chromosomes (Table 5), while the average number of I’s, II’s, III’s, and IV’s was 8.2, 20.4, 0.2, and 0.7, respectively. A few lagging chromosomes were also observed at Anaphase-I. The high frequency of univalents (5–12) and multivalents (0–1) caused meiotic disturbance; the plants were partially fertile/sterile.

**G. hirsutum × G. arboreum.** The plant progenies of this combination comprised 12 plants; cytological studies were conducted on six normal morphological plants with better boll setting. The cytological configuration at Metaphase-I of the BC1 plants revealed two I’s, 23 II’s, and one IV’s (Figure 6E). In the 53 PMCs of these hybrid plants, there were 1–5 I’s, 20–25 II’s, and 0–1 III’s and IV’s, for a total of 52 chromosomes (Table 5), while the average number of I’s, II’s, III’s, and IV’s for 53 PMCs was 2.2, 23.2, 0.4, and 0.6, respectively. Although multivalent association was observed, the high frequency of bivalents (20–25) caused these plants to be fertile or partially fertile.
Meiosis in BC2: 2(G. arboreum) × G. hirsutum.

These plant progenies consisted of 14 plants; only five normal morphological plants with better boll setting were studied cytologically. The chromosomal conformation at Metaphase-I was two I’s + 23 II’s + 1 IV’s (Figure 6F). A study of 60 PMCs revealed 2–4 I’s, 23–25 II’s, and 0–1 IV’s, for a total of 52 chromosomes (Table 5), while the average number of I’s, II’s, and IV’s for 60 PMCs was 3.0, 24.1, and 0.2, respectively. Trivalents were not observed in these plants. The low frequency of uni- and multi-valents, as well as the high frequency of chromosome

| Table 3. Morphological characteristics of parents and the BC1 to BC3 progenies from the cross 2(G. arboreum) × G. hirsutum. |
|--------------------------------------------------|--|--|--|--|--|
| Morphological characteristic | 2(G. arboreum) | G. hirsutum | BC1 | BC2 | BC3 |
| Stem characteristics | | | | | |
| Stem color | Greenish brown | Green | Brown | Brown | Brown |
| Stem hairiness | Profusely hairy | Hairy | Hairy | Hairy | Hairy |
| Black glands | Dense | Sparse | Sparse | Sparse | Sparse |
| Leaf characteristics | | | | | |
| Leaf color | Dark green | Green | Light/dark green | Light/dark green | Light/dark green |
| Leaf size(cm) | Medium (7.3×9.9) | Large (10×14) | Small/Large (7.0×8.0 cm)/(9.0×11.0 cm) | Small/Large (7.1×8.3)/(9.2×10.8) | Medium/Large (7.6×8.9)/(9.6×11.4) |
| Leaf hairiness | Profusely hairy | Hairy | Hairy/profusely hairy | Hairy/profusely hairy | Hairy/profusely hairy |
| Leaf lobation | 3–5 narrow, deep lobed | 3–5 broad, shallow lobed | 3–5 broad lobed | 3–5 broad lobed | 3–5 broad lobed |
| Leaf texture | Thick, Leathery | Herbaceous | Herbaceous | Herbaceous | Herbaceous |
| Petiole length (cm) | Medium (4.4) | Long (8.8) | Long (7.3) | Long (7.2) | Long (7.5.0) |
| Boll characteristics | | | | | |
| Bracteole number and size (cm) | 2–3, large (3.0×2.6), united at base | 3 large (3.3×1.8) | 3 Large (3.0×2.3) | 3 Large (3.3×2.2) | 3 Large (3.1×2.4) |
| Bracteole dentation | Entire | 5–11, deep narrow | 4–9 medium | 3–10 medium | 3–11 medium |
| Flower characteristics | | | | | |
| Flower size | Medium | Large | Medium | Medium | Medium |
| Pedicel size (cm) | Long (1.7) | Long (1.2) | Long (1.3) | medium (1.0) | medium (0.9) |
| Calyx | 5 sepal forming a cup with wavy margins | 5 sepal forming a cup with wavy margins | 5 sepal forming a cup with wavy margins | 5 sepal forming a cup with wavy margins |
| Corolla color | Light yellow | Creamy | Creamy/light yellow | Creamy/light yellow | Creamy/light yellow |
| Petal number and size (cm) | 5, medium (3.0×2.6) | 5, large (4.6×4.5) | 5, medium (3.5×4.1) | 5, medium (3.4×4.2) | 5, medium (3.3×3.5) |
| Petal spot | Dark pink | Absent | Present/absent | Present/absent |
| Position of staminal column and size (cm) | Short (0.4) | Long (2.0) | Medium (1.5) | Medium (1.7) | Medium (1.6) |
| Anther dehiscence | Partial | Normal | Partial | Normal | Normal |
| Pollen color | Light yellow | Creamy | Yellow | Creamy/light yellow | Creamy/light yellow |
| Pistil size (cm) | Long (2.5) | Long (2.9) | Long (2.6) | Long (2.8) | Long (2.9) |

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association (23–25 II’s), caused the plants to be fertile but shy bearing.

**G. hirsutum × G. arboreum.** The plant progenies of this combination comprised 161 plants; only 10 normal morphological plants with better boll setting were studied cytologically. The chromosomal constitution at Metaphase-I revealed 3 I’s+21 II’s+1 III’s+1 IV’s (Figure 6G). However, in 110 PMCs, there were 1–4 I’s, 21–25 II’s, and 0–1 III’s and IV’s, for a total of 52 chromosomes (Table 5), and the average number of I’s, II’s, III’s, and IV’s was 2.4, 23.2, 0.4, and 0.5, respectively. Low frequencies of univalents (1–4) and multivalents (0–1), as well as high frequencies of bivalents (21–25), were observed. The plants were fertile. A few shy bearing plants were also observed.

**Meiosis in BC3.** [2(G. arboreum) × G. hirsutum]. The plant progenies consisted of 12 plants. A total of 35 PMCs were sampled from two plants for microscopic studies. Metaphase-I of these PMCs showed 2 I’s+25 II’s (Figure 6H). The average range of these PMCs revealed that there were 2 I’s and 25 II’s, for a total of 52 chromosomes (Table 5); the plants were fertile.

**G. hirsutum × G. arboreum.** The plant progenies consisted of 225 plants. The chromosome pairing was normal (26 II’s) in most of the PMCs (Figure 6I). The average number of chromosomes among 40 PMCs exhibited normal disjunction (Table 5); the plants were fertile.

**Testing of BC1 to BC3 progenies against CLCuD under natural field conditions**

The BC1 to BC3 hybrid plants of 2(G. arboreum) × G. hirsutum and G. hirsutum × G. arboreum were tested under natural field conditions. Nineteen plants of BC1 of the combination 2(G. arboreum) × G. hirsutum and 15 plants of reciprocal cross G. hirsutum × G. arboreum revealed disease indices of 1.3% and 1.6%, respectively, whereas the average severity index was 0.05 and 0.06 at 40 DAS, respectively (Table 7). However, the disease index and severity index were zero after 70 DAS because the minor spots of vein thickening that were observed on a single plant of each cross disappeared after 70 DAS. CIM 496, the control variety used in this trial, had a disease index of 94.3%, and enation was also observed at 70 DAS. Twenty-one plants of BC2 derived from the combination 2(G. arboreum) × G. hirsutum and 161 plants of the combination G. hirsutum × G. arboreum raised through backcrossing of BC1 with G. hirsutum had disease indices of 1.8% and 4.0%, respectively, at 40 DAS, and the disease index increased to 3.5% and 6.8%, respectively, at 70 DAS. The grade of disease severity in 2(G. arboreum) × G. hirsutum was 0.07 (40 DAS) to 0.1 (70 DAS), whereas it was 0.17 (40 DAS) to 0.2 (70 DAS) for G. hirsutum × G. arboreum. The susceptible cotton variety CIM 496 in this trial had a disease index of 97.7% with a disease severity grade of 3.9.

Twelve plants of BC3 of the combination 2(G. arboreum) × G. hirsutum and 225 plants of the combination G. hirsutum × G. arboreum raised through backcrossing with G. hirsutum had a 4.2% and 7.0% disease index, respectively, at 40 DAS. And the
disease index increased to 8.3% and 12.0%, respectively, at 70 DAS. The grade of disease severity in 2(G. arboreum)6G. hirsutum was 0.17 (40 DAS) to 0.3 (70 DAS), whereas it was 0.28 (40 DAS) to 0.5 (70 DAS) for G. hirsutum6G. arboreum. CIM 496 had a disease index of 95.0% with a disease severity grade of 3.8 (Table 7).

As the backcross progressed from BC1 to BC3, the PDT gradually decreased (Figure 8). However, the PDT was fairly high in 2(G. arboreum)6G. hirsutum compared to the combination G. hirsutum6G. arboreum.

Discussion

G. hirsutum has low genetic diversity and lacks resistance against CLCuD. In general, wild diploid species of Gossypium possess resistance against many challenges, such as insects, pests, diseases, and many abiotic factors [37,46]. Hence, there is a great need to exploit this resource to develop resistance against CLCuD in cultivated tetraploid species [2]. Cotton breeders have long tried to obtain hybrids between diploid and tetraploid species [47]. However, several incompatibility factors hinder the development

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**Table 4.** Morphological characteristics of parents and the BC1 to BC3 progenies from the cross G. hirsutum × G. arboreum.

| Morphological characteristics | G. hirsutum | G. arboreum | BC1 | BC2 | BC3 |
|-------------------------------|-------------|-------------|-----|-----|-----|
| **Stem characteristics**     |             |             |     |     |     |
| Stem color                    | Green       | Green       | Green | Green | Green |
| Stem hairiness                | Hairy       | Hairy       | Hairy | Hairy | Hairy |
| Black glands                  | Sparse      | Sparse/dense| Sparse | Sparse | Sparse |
| **Leaf characteristics**      |             |             |     |     |     |
| Leaf color                    | Green       | Green       | Green | Green/dark green | Green/dark green |
| Leaf size (cm)                | Large (10.0 × 14.0) | Small/medium (6.0 × 8.3) | Medium (7.1 × 7.9) | medium (7.0 × 8.4) | medium (7.3 × 8.3) |
| Leaf lobation                 | 3–5 broad, shallow lobed | 3–5 narrow, deep lobed | 3–5 medium lobed | 3–5 broad lobed | 3–5 broad lobed |
| Leaf texture                  | Herbaceous  | Herbaceous  | Herbaceous | Herbaceous | Herbaceous |
| Leaf hairiness                | Hairy       | Hairy/profusely hairy | Hairy | Hairy/profusely hairy | Hairy/profusely hairy |
| Petiole length (cm)           | Long (8.8) | Medium (4.4) | Long (7.2) | Long (7.5) | Long (7.4) |
| **Boll characteristics**      |             |             |     |     |     |
| Bracteole number and size (cm)| 3 large (3.3 × 1.8) | 3, small (2.7 × 2.1), united at base | 3, large (3.0 × 2.6) | 3, large (3.0 × 2.0) | 3, large (3.2 × 2.2) |
| Bracteole dentation           | 3–7, superficial | 5–11, deep narrow | 4–9, superficial | 3–11, superficial | 3–9, superficial |
| **Flower characteristics**    |             |             |     |     |     |
| Flower size                   | Large       | Small       | Medium | Medium | Medium/large |
| Pedicel size (cm)             | Long (1.2) | Long (1.2) | Long (1.1) | Long (1.3) | Long (1.2) |
| Calyx                         | 5 sepal forming a cup with teeth | 5 sepal forming a cup with wavy margins | 5 sepal forming a cup with wavy margins | 5 sepal forming a cup with wavy margins | 5 sepal forming a cup with wavy margins |
| Corolla color                 | Creamy      | Yellow      | Creamy/Light Yellow | Creamy/light yellow | Creamy/light yellow |
| Petal number and size (cm)    | 5, large, (4.6 × 4.5) | 5, small (2.6 × 2.5) | 5, large, (4.5 × 4.4) | 5, large, (4.4 × 4.6) | 5, large, (4.6 × 4.4) |
| Petal spot                    | Absent      | Light pink  | Present/absent | Present/absent | Present/absent |
| Position of staminalcolum and size (cm) | Long (2.0) | small (1.0) | Medium (1.5) | Medium (1.5) | Medium (1.6) |
| Anther dehiscence             | Normal      | Normal      | Partial/normal | Normal | Normal |
| Pollen color                  | Creamy      | Yellow      | Creamy/Light Yellow | Creamy/light Yellow | Creamy/light Yellow |
| Pistil size (cm)              | Long (2.9) | Small (2.1) | Long (3.1) | Long (2.9) | Long (3.2) |

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Figure 5. Hybrid Progenies of (G. hirsutum × G. arboreum). A = BC1; B, C = BC2; D = BC3.

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Figure 6. Chromosome configurations in PMCs at Metaphase-I of meiosis. A. G. hirsutum, 26 II's; B. G. arboreum, 13 II's; C. 2(G. arboreum), 2 I's+23 II's+1 IV; D. [2(G. arboreum) × G. hirsutum] BC1, 6 I's+21 II's+1 IV; E. [G. hirsutum × G. arboreum] BC1, 2 I's+23 II's+1 IV; F. [2(G. arboreum) × G. hirsutum] BC2, 2 I's+23 II's+1 IV; G. [G. hirsutum × G. arboreum] BC2, 3 I's+21 II's+1 III+1 IV; H. [2(G. arboreum) × G. hirsutum] BC3, 2 I's+25 II's; I. [G. hirsutum × G. arboreum] BC3, 26 II's.

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Table 5. Cytological comparison of BC₁ to BC₃ plants from an interspecific cross between *G. arboreum* and *G. hirsutum*.

| Cross configuration | Plant number | PMC | I’s | II’s | III’s | IV’s | Total |
|---------------------|--------------|-----|-----|------|-------|------|-------|
| **Chromosomal configuration for BC₁** | | | | | | | |
| 2(*G. arboreum*) × *G. hirsutum* | P2 | 12 | 5 | 20 | 1 | 1 | 52 |
| // | P3 | 10 | 6 | 21 | 0 | 1 | 52 |
| // | P4 | 8 | 6 | 21 | 0 | 1 | 52 |
| // | P9 | 10 | 8 | 20 | 0 | 1 | 52 |
| // | P11 | 15 | 8 | 22 | 0 | 0 | 52 |
| // | P13 | 11 | 12 | 20 | 0 | 0 | 52 |
| // | P4 | 10 | 12 | 18 | 0 | 1 | 52 |
| Range | 5–12 | 18–22 | 0–1 | 0–1 | |
| Average of 76 cells | 8.2 | 20.4 | 0.2 | 0.7 | |
| *G. hirsutum* × *G. arboreum* | P1 | 5 | 2 | 25 | 0 | 0 | 52 |
| // | P2 | 12 | 1 | 22 | 1 | 1 | 52 |
| // | P5 | 8 | 5 | 20 | 1 | 1 | 52 |
| // | P9 | 10 | 2 | 23 | 0 | 1 | 52 |
| // | P10 | 6 | 2 | 25 | 0 | 0 | 52 |
| // | P11 | 12 | 2 | 25 | 0 | 0 | 52 |
| Range | 1–5 | 20–25 | 0–1 | 0–1 | |
| Average of 53 cells | 2.2 | 23.2 | 0.4 | 0.6 | |
| **Chromosomal configuration for BC₂** | | | | | | | |
| 2(*G. arboreum*) × *G. hirsutum* | P3 | 15 | 4 | 24 | 0 | 0 | 52 |
| // | P4 | 12 | 2 | 25 | 0 | 0 | 52 |
| // | P7 | 8 | 2 | 25 | 0 | 0 | 52 |
| // | P10 | 12 | 2 | 23 | 0 | 1 | 52 |
| // | P11(1) | 15 | 4 | 24 | 0 | 0 | 52 |
| Range | 2–4 | 23–25 | 0 | 0–1 | |
| Average of 60 cells | 3.0 | 24.1 | 0 | 0.2 | |
| (*G. hirsutum*) × *G. arboreum* | P1(16) | 15 | 1 | 24 | 1 | 0 | 52 |
| P2 | 10 | 3 | 23 | 1 | 0 | 52 |
| // | P4(1) | 12 | 2 | 25 | 0 | 0 | 52 |
| // | P5(3) | 10 | 2 | 25 | 0 | 0 | 52 |
| // | P5(14) | 8 | 4 | 22 | 0 | 1 | 52 |
| // | P7(15) | 5 | 3 | 23 | 1 | 0 | 52 |
| // | P1(15) | 10 | 2 | 23 | 0 | 1 | 52 |
| // | 10 | 2 | 23 | 0 | 1 | 52 |
of hybrids under in situ conditions [48,49]. Abortion of the embryo after fertilization and the lack of retention of cross bolls [50,51] is a common stumble in interspecific crosses. Some species like G. barbadense can be hybridize easily with G. hirsutum and produce fertile F1 progeny [52] without hormones application. These two species i.e. G. hirsutum and G. barbadense have chromosome homology and the tetraploid genomes, are not separated by any large scale chromosomal rearrangement [53]. However, crosses between G. hirsutum and G. arboreum L. are rarely successful without hormone application [44,54]. Plant hormones are known to control pollen tube growth [55].

Exogenous application of growth hormones has been used to overcome the crossing barrier and to facilitate interspecific crosses in many crops, i.e., cotton [36], wheat [57], and tomato [58]. Altman [56] compared exogenous application with in vitro techniques, i.e., ovule and embryo culture, and found that exogenous hormone application in conjunction with standard hybridization methods is superior to in vitro methods. Interspecific hybridization of cotton is enhanced by the application of exogenous hormones after pollination. Exogenous hormone application alone may be used to overcome certain crossing barriers within Gossypium [59–60]. The extract of garlic acid has been used as a growth regulator to obtain interspecific hybrids between tetraploid G. hirsutum and diploid G. arboreum species of cotton [56,61].

The boll setting and seed germination is very low in interspecific crosses and fertility of interspecific crosses can be measured in terms of boll setting percentage [52,62]. The cross fertility of BC1 to BC3 between [2(G. arboreum)×G. hirsutum] and G. hirsutum×G. arboreum showed that the boll set was maximum (42.9%) in cross BC3, G. hirsutum×G. arboreum, but minimum (1.3%) in cross BC2, 2(G. arbo-rum)×G. hirsutum (Table 2). Viable seeds were obtained in both combinations. From BC2 to BC3, an increasing trend of boll setting and germination (%) was observed. Seed setting improvement was also recorded in Brassica by backcrossing with the recurrent parent [63]. The factor responsible for the semi-sterile condition are transmitted rarely through the pollen but readily through the egg cell. Boll setting and germination (%) was higher in reciprocal cross (G. hirsutum×G. arboreum) as compared to direct cross 2(G. arboreum)×G. hirsutum [64].

In general, the BC1 to BC3 hybrid plants of both cross combinations [2(G. arboreum)×G. hirsutum and G. hirsutum×G. arboreum] were intermediate in several traits between the two parents. The prevalence of yellow pollen in both crosses (direct and reciprocal) in most of the plants validated the inheritance of this character from G. arboreum, because this color is more common in G. arboreum species [65,66], and it revealed the dominance in inheritance [67]. By contrast, in BC1 to BC3, leaf hairiness, flower size, corolla color, petal spots, pollen color, and so on were segregated in both parents. Moreover, morphological characteristics particularly leaf texture, leaf size, bracteole size, corolla color and petal spots from G. arboreum into BC1 to BC3 progenies [68] of both crosses were helpful for selection of plant progenies that have resemblance to G. arboreum and also showed CLCuD resistance. The frequency of plant progenies that showed

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**Table 5. Cont.**

| Chromosomal configuration for BC3 | Pmc | I’s | II’s | III’s | Total |
|----------------------------------|-----|-----|-----|-------|-------|
| 2(G. arboreum)×G. hirsutum       | 15  | 2   | 25  | 0     | 52    |
| G. hirsutum×G. arboreum          | 20  | 2   | 25  | 0     | 52    |

Range 0–26 0–0

Average of 40 cells 0 26 0 0

---

**Table 5.**

| Chromosomal configuration for BC4 | Pmc | I’s | II’s | III’s | Total |
|----------------------------------|-----|-----|-----|-------|-------|
| 2(G. arboreum)×G. hirsutum       | 15  | 2   | 25  | 0     | 52    |
| G. hirsutum×G. arboreum          | 20  | 2   | 25  | 0     | 52    |

Range 0–26 0–0

Average of 40 cells 0 26 0 0

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The boll setting and seed germination of hybrids under in situ conditions [48,49]. Abortion of the embryo after fertilization and the lack of retention of cross bolls [50,51] is a common stumble in interspecific crosses. Some species like G. barbadense can be hybridize easily with G. hirsutum and produce fertile F1 progeny [52] without hormones application. These two species i.e. G. hirsutum and G. barbadense have chromosome homology and the tetraploid genomes, are not separated by any large scale chromosomal rearrangement [53]. However, crosses between G. hirsutum and G. arboreum L. are rarely successful without hormone application [44,54]. Plant hormones are known to control pollen tube growth [55].

Exogenous application of growth hormones has been used to overcome the crossing barrier and to facilitate interspecific crosses in many crops, i.e., cotton [36], wheat [57], and tomato [58]. Altman [56] compared exogenous application with in vitro techniques, i.e., ovule and embryo culture, and found that exogenous hormone application in conjunction with standard hybridization methods is superior to in vitro methods. Interspecific hybridization of cotton is enhanced by the application of exogenous hormones after pollination. Exogenous hormone application alone may be used to overcome certain crossing barriers within Gossypium [59–60]. The extract of garlic acid has been used as a growth regulator to obtain interspecific hybrids between tetraploid G. hirsutum and diploid G. arboreum species of cotton [56,61]. The **in situ** development of BC1 to BC3 plants using exogenous hormones in the current study was superior to that using in vitro methods, which is in agreement with an earlier report [55]. The average number of seeds per boll varied from immature seeds to 1.5 seeds per boll. In the absence of exogenous hormones, pollinated flowers produce 0.1% seed development [56].
good plant architecture were higher in reciprocal crosses as compared to direct cross.

When developing interspecific hybrids for resistance, a thorough knowledge of the chromosomal behavior in hybrids and backcross progenies is essential. In the present study, in hybrid 2(G. arboreum)×G. hirsutum, the ‘AD’ genome was introgressed into the ‘AA’ genome of G. arboreum, producing an ‘AAAD’ genomic constitution. In hybrid G. hirsutum×G. arboreum, the A-genome of G. arboreum, producing the genomic constitution ‘AAD’. In G. arboreum and G. hirsutum, normal orientation, association, and disjunction of chromosomes were observed, while in F1 hybrids of the above genomic constitution, quadrivalents and a low frequency of chromosome association (bivalents) were observed. The univalents observed in this study can be attributed to asynapsis due to the lack of homology between the different sets of chromosomes. The presence of laggards demonstrates the occurrence of meiotic disturbances, leading to an imbalance in the daughter cells. In BC1 hybrid plants of both combinations, the frequency of univalents and multivalents was high, and the plants were sterile/partially fertile. In BC2 hybrids of both combinations, the frequency of univalents and multivalents was low, and the plants were shy bearing. In BC3 hybrids of both combinations, the frequency of chromosome association (bivalents) was 25–26; hence, the plants were fertile. The average of univalent (I’s) chromosomes was higher in 2(G. arboreum)×G. hirsutum in comparison with G. hirsutum×G.arboreum. However, the average of bivalents (II’s) chromosomes was higher in G. hirsutum×G.arboreum. Thus G. hirsutum×G.arboreum was more fertile and more adaptive to the environment than 2(G.arboreum)×G. hirsutum.

Studies of resistance/susceptibility are rather difficult and laborious due to the involvement of vectors, the efficiency of transmission, and the persistent nature of the virus/CLCuD. Grafting may successfully lead to the transmission of the virus when other methods fail, as it involves the union of cambial layers of the root sock and scion [69–70]. Thus, to screen CLCuD-resistant germplasm, transmission by grafting is the best alternative to natural transmission by vector, as most viruses of a persistent nature, such as CLCuD, cannot be transmitted through mechanical inoculation [71]. Ahmad et al. [72] used sick plot techniques to screen the exotic and local germplasm against CLCuD.

The BC1 to BC3 of 2(G. arboreum)×G. hirsutum and G. hirsutum×G. arboreum were tested through grafting under natural field/greenhouse conditions. These hybrids remained resistant to CLCuD [39]. The results of evaluation of the BC1 to BC3 progenies revealed a high degree of variability for CLCuD in the field and through grafting. All plants from both crosses [2(G. arboreum)×G. hirsutum and G. hirsutum×G. arboreum] showed 100% infectivity and grafting success. However, latent period and infection type range for BC1–BC2 was better in 2(G.arboreum)×G. hirsutum cross than G. hirsutum×G.arboreum. The grafts of BC1 from cross 2(G.arboreum)×G. hirsutum remained asymptomatic to this disease. However, BC1 of G. hirsutum×G. arboreum showed minor vein thickening, but the vein thickening was highly reduced after 70 days of grafting [46], whereas CIM-496 showed symptoms of CLCuD within 11–14 days after germination. Although minor symptoms of CLCuD appeared in BC1 of G. hirsutum×G. arboreum, this disease did not affect the growth of the plants. Therefore, we can conclude that these plants were also resistant to CLCuD. The BC2 and BC3 hybrid plants of both cross combinations 2(G. arboreum)×G. hirsutum and G. hirsutum×G. arboreum developed disease symptoms after 28–35 DAG, and the average disease severity was grade 1.0 (70 DAG). Additionally, these plants showed good tolerance to CLCuD, with no symptoms of stunted growth. Therefore, these BC2 and BC3 plants were highly tolerant to CLCuD compared with susceptible variety CIM-496, which showed CLCuD symptoms after 11 DAG with no boill setting. Ullah et al. [46] also observed mild symptoms of CLCuD on the introgressed material following grafting, but the amount of viral DNA was significantly lower than the levels found in G. hirsutum. The same trend/response for latent period to acquire CLCuD was observed in the field for BC1 to BC3 hybrid plants. The average severity index for 2(G. arboreum)×G. hirsutum and G. hirsutum×G. arboreum was 0.05 and 0.06 (40 DAG), respectively. However, the disease index and severity index were zero after 70 DAG. Thus, the resistant hybrid plants of both crosses 2(G. arboreum)×G. hirsutum and G. hirsutum×G. arboreum showed better tolerance to CLCuD, with no deleterious effects on yield or growth. However, 2(G. arboreum)×G. hirsutum plants were more tolerant regarding number of virus infected plants, disease index (%), severity index and infection type range than those of cross G. hirsutum×G. arboreum. Collectively, plants from these crosses had better tolerance to CLCuD than CIM-496. The PDT was higher in 2(G. arboreum)×G. hirsutum than in G. hirsutum×G. arboreum. The frequency of ideotype plants was higher in G. hirsutum×G. arboreum compared with 2(G. arboreum)×G. hirsutum.

Conclusions
The results indicate that the BC1 to BC3 progenies were highly tolerant to CLCuD, indicating the possibility of transferring...
### Table 6. Evaluation of plants from an interspecific cross between *G. arboreum* and *G. hirsutum* against cotton leaf curl virus disease through grafting.

| Progeny | Year | No. of plants tested | Grafting success (%) | Infectivity (%) | Latent period (days) | Infection type range<sup>a</sup> | Av disease severity after 70 (DAG) | Disease reaction |
|---------|------|----------------------|----------------------|-----------------|---------------------|------------------------------|-------------------------------|----------------|
| BC<sub>1</sub> | 2011 | 15                   | 100                  | 100             | Symptomless         | 0                           | 0                             | Resistant       |
|         |      |                      |                      |                 |                     |                              |                               |                 |
| G. hirsutum × *G. arboreum* | 2011 | 12                   | 100                  | 100             | 39–41               | 0–1                         | 1                             | Highly tolerant |
| CIM-496 (Std.) | 2011 | 20                   | 100                  | 100             | 14                  | 3–4E<sup>*</sup>             | 4E                           | Highly susceptible |
| BC<sub>2</sub> | 2012 | 14                   | 100                  | 100             | 30–35               | 0–1                         | 1                             | Highly tolerant |
| G. hirsutum × *G. arboreum* | 2012 | 20                   | 100                  | 100             | 28–30               | 0–2                         | 1                             | Highly tolerant |
| CIM-496 (Std.) | 2012 | 10                   | 100                  | 100             | 11                  | 3–4E<sup>*</sup>             | 4E                           | Highly susceptible |
| BC<sub>3</sub> | 2013 | 12                   | 100                  | 100             | 28–30               | 0–2                         | 1                             | Highly tolerant |
| G. hirsutum × *G. arboreum* | 2013 | 16                   | 100                  | 100             | 25–30               | 0–3                         | 1                             | Highly tolerant |
| CIM-496 (Std.) | 2013 | 10                   | 100                  | 100             | 11                  | 3–4E<sup>*</sup>             | 4E                           | Highly susceptible |

<sup>a</sup>Infection type range is based on the 0–4 scale described in Table 1; *Enation where observed.

### Table 7. Evaluation of plants from an interspecific cross between *G. arboreum* and *G. hirsutum* against cotton leaf curl virus disease under natural field conditions.

| Parentage | No. of plants tested | Latent period (days) | No. of virus infected plants | Disease index (%) | Severity index | Infection type range<sup>a</sup> | Disease reaction<sup>b</sup> |
|-----------|----------------------|----------------------|------------------------------|-------------------|---------------|-------------------------------|--------------------------|
| BC<sub>1</sub> | 19                   | Symptomless          | 19(18<sup>+</sup>1<sup>+</sup>) | 19(19<sup>+</sup>) | 1.3           | 0.05                          | 0                        | 0                        | 0                        | R                        |
| G. hirsutum × *G. arboreum* | 15                  | 35–40                | 15(14<sup>+</sup>1<sup>+</sup>) | 15(15<sup>+</sup>) | 1.6           | 0.06                          | 0                        | 0–1                      | 0                        | R                        |
| CIM-496 (Std.) | 31                  | 14                   | 31(1<sup>+</sup>3<sup>+</sup>2<sup>+</sup>5<sup>+</sup>) | 31(4<sup>+</sup>2<sup>+</sup>7<sup>+</sup>) | 94.35         | 96.7E<sup>*</sup>            | 3.7                      | 3<sup>+</sup>4E         | 3<sup>+</sup>4E            | HS                       |
| BC<sub>2</sub> | 14                   | 30–35                | 14(13<sup>+</sup>1<sup>+</sup>) | 14(12<sup>+</sup>2<sup>+</sup>) | 1.8           | 3.5                           | 0.07                     | 0.1                      | 0 – 1                    | HT                       |
| G. hirsutum × *G. arboreum* | 161                 | 25–30                | 161(146<sup>+</sup>7<sup>+</sup>5<sup>+</sup>3<sup>+</sup>) | 161(138<sup>+</sup>9<sup>+</sup>8<sup>+</sup>5<sup>+</sup>1<sup>+</sup>) | 4             | 6.8                           | 0.17                     | 0.2                      | 0 – 3                    | HT                       |
| CIM-496 (Std.) | 168                 | 13                   | 168(4<sup>+</sup>2<sup>+</sup>7<sup>+</sup>1<sup>+</sup>57<sup>+</sup>) | 168(4<sup>+</sup>11<sup>+</sup>1<sup>+</sup>53<sup>+</sup>) | 97.7          | 97.1                           | 3.9                      | 3<sup>+</sup>4E        | 2<sup>+</sup>4E            | HS                       |
| BC<sub>3</sub> | 12                   | 25–30                | 12(11<sup>+</sup>1<sup>+</sup>) | 12(9<sup>+</sup>2<sup>+</sup>1<sup>+</sup>) | 4.2           | 8.3                           | 0.17                     | 0.3                      | 0 – 2                    | HT                       |
| G. hirsutum × *G. arboreum* | 225                 | 25–30                | 225(200<sup>+</sup>5<sup>+</sup>1<sup>+</sup>8<sup>+</sup>6<sup>+</sup>6<sup>+</sup>) | 225(176<sup>+</sup>1<sup>+</sup>4<sup>+</sup>12<sup>+</sup>1<sup>+</sup>9<sup>+</sup>) | 7             | 12                             | 0.28                     | 0.5                      | 0 – 4                    | HT                       |
| CIM-496 (Std.) | 190                 | 15                   | 190(4<sup>+</sup>6<sup>+</sup>1<sup>+</sup>180<sup>+</sup>) | 190(6<sup>+</sup>15<sup>+</sup>169<sup>+</sup>) | 95            | 96.4                           | 3.8                      | 3.8                      | 2<sup>+</sup>4E              | 2<sup>+</sup>4E            | HS                       |

<sup>a</sup>Infection type range is based on the 0–4 scale described in Table 1; *Enation where observed; R Resistant; HT Highly tolerant; HS Highly susceptible; <sup>b</sup>Disease reaction based on disease index 70 DAS.

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BC1 (1.3–1.6%) to BC2 (1.8–4.0%) to BC3 (4.2–7.0%). However, the disease incidence was much lower than that of the commercial cultivar CIM-496, which exhibited a very high incidence of CLCuD (97.7%). The disease incidence was lower in combination 2(G. arboreum)×G. hirsutum than in G. hirsutum×G. arboreum. As “A” genome is an invaluable genetic resource for improving modern tetraploid cotton (G. hirsutum). We observed very wide genetic variability among BC1 to BC3 progenies, which will certainly facilitate improvement of cotton resistances to diseases.

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Author Contributions
Conceived and designed the experiments: WN SA. Performed the experiments: ALT KM. Analyzed the data: BZ. Contributed reagents/materials/analysis tools: BZ SA. Contributed to the writing of the manuscript: WN BZ SA. Provided the breeding material: AM SA.

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