The Journal of Animal & Plant Sciences, 23(5): 2013, Page: 1436-1440
ISSN: 1018-7081

GOSSYPIUM GOSSYPIOIDES: A SOURCE OF RESISTANCE AGAINST COTTON LEAF CURL DISEASE AMONG D GENOME DIPLOID COTTON SPECIES

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ABSTRACT

The diploid cotton species belonging to A, D and tetraploids with AD genome were screened for the presence/absence of components of cotton leaf curl complex. These cotton species are being maintained in a living herbarium for more than fourdecades under natural conditions. As expected, the two diploid species (*Gossypium arboreum* and *G. herbaceum*) of A genome, were found to be free of virus when screened by PCR and Φ29 DNA polymerase. The two cotton species of D genome (*G. thurberii* and *G. aridum*) showed the presence of begomovirus. The species belonging to AD genome were found to be susceptible to CLCuD due to the presence of both begomovirus and betasatellite. However, the interesting exception among D genome species was *G. gossypioides*. The results from present study suggest that *G. gossypioides* is a valuable resource for mapping of resistance by developing segregating populations and for developing synthetic tetraploids.

Key words: Resistance, susceptibility, Cotton leaf curl disease, betasatellite.

INTRODUCTION

Cotton belongs to genus *Gossypium*, which is comprised of more than 50 species including wild as well as cultivated. Among them 45 are diploid and five are allotetraploid in nature (Fryxell, 1979). These wild species are divided into eight genomes from A through G and K. There are four cultivated species of cotton that include *Gossypium hirsutum* (cultivated species, allotetraploid, AD_1), *G. barbadense* (cultivated species, allotetraploid, AD_2), *G. arboreum* (cultivated, diploid, A_2) and *G. herbaceum* (cultivated, diploid, A_1) originated from South Mexico, South America (Peru), Indian sub-continent and South Africa, respectively. The two tetraploid cotton species that originated in the New World now contribute more than 90% of cotton produced in the world. The wild species of cotton are potential source of resistance to biotic (insects and diseases) and abiotic stresses like salinity, cold, drought, heat (Yik and Birchfield, 1984, Narayanan and Singh, 1994). Some of wild species namely, *G. darwinii*, *G. stocksii*, *G. harkensii*, *G. aridum*, are good donors for drought tolerance whereas *G. thurberii* has resistant genes for frost tolerance (Rooney *et al*., 1991). Due to their potential, these wild species (diploids and tetraploids) are being utilized in various hybridization programs (Mehetre *et al*., 2003, Mehetre *et al*., 2004).

Cotton leaf curl disease (CLCuD) is the most important limiting factor for cotton production in Pakistan and bordering areas of India (Briddon and Markham, 2000). The disease is caused by monopartite begomoviruses that require a disease-specific DNA satellite named Cotton leaf curl Multan betasatellite (CLCuMB) (Briddon *et al*., 2001; Mansoor *et al*., 2003). The betasatellite is a symptom determinant and is essential for the induction of disease symptoms (Qazi *et al*., 2007) and DNA A encodes various genes required for replication (Hanley-Bowdoin *et al*., 1999) and coat protein that play important role in disease transmission (Azzam *et al*., 1994). The first epidemic of CLCuD started in early 1990s (known as "Multan strain") was managed by the introduction of resistant varieties developed through conventional breeding. However, in 2001 symptoms of the disease started to appear on previously resistant varieties due to emergence of "Burewala strain" (Amrao *et al*., 2010). The breakdown of natural resistance in *G. hirsutum* introgressed from LRA5166 and CP15/2 has further enhanced interest and efforts to utilize resistance from diploid species through interspecific hybridization. *G. arboreum* is still grown commercially and has consistently shown resistance against both "Multan strain" and "Burewala strain" of CLCuD. Surveys on large number of plants of *G. arboreum* did not identify even a single plant showing symptoms of CLCuD (Briddon and Markham, 2000). One of the largest living herbarium of wild species of *Gossypium* is being maintained at Central Cotton Research Institute (CCRI), Multan, Pakistan. These wild species are being maintained since 1970s and are growing in an area with inoculum of CLCuD and other begomoviruses (Azhar *et al*., 2010). No information is available in the literature about the contribution diploid wild species for resistance to CLCuD. Efforts have been

made in the present study to investigate the contributor of resistance/susceptibility to viruses in tetraploid cotton species or diploid species belong to A or D genome. The detailed information about sources of resistance would be useful to cotton breeders to make inter-specific hybrids.

MATERIALS AND METHODS

The genomic DNA was isolated by Cetyl triethyl methyl ammonium bromide method (Doyle and Doyle, 1990) from twenty randomly collected leaf tissues from each cotton species including *G. arboreum*, *G. herbaceum*, *G. thurberii*, *G. herkensii*, *G. aridum*, *G. gossypioides*, *G. hirsutum*, *G. mustelinum* and *G. darwanii*. These species are grown and maintained for the last four decades at CCRI, Multan, Pakistan (Fig 1) and this living herbarium is exposed to CLCuD. Diagnostic primers, Begomo-F (ACGCGT GCCGTGCTGCTGCCCCCATTGTCC) and Begomo-R (ACGCGTATGGGCTGYCGAAGTTSAGAC); β01 (GGTACCACTACGCTACGCAGCAGCC) and β02 (GGTACCTACCCTCCCAGGGGTACA) were used to amplify begomoviruses and betasatellites, respectively (Briddon *et al*., 2001). For each polymerase chain reaction (PCR), 3 µl of 5 ng/µl diluted DNA (dilution was made after quantification at 260 nm, using spectrophotometer, Smart Spec Plus (Spectrophotometer, Bio-Rad), 3 to 4 units of *Taq* Polymerase, 10X *Taq* buffer with 5 µl of $(NH4)_2SO_4$, 3 µl of 25 mM $MgCl_2$, 5 µl of 2 mM dNTPs, 1 µl of 10 pmole of primer and 29.5 µl of $d2H_2O$, to make final volume of 50 µl, and mixed in PCR tubes of 0.25 ml. The PCR profile was set at initial cycle for denaturation of DNA duplex at 94°C for 5 minutes followed by 35 cycles, each consisting of 30 seconds for denaturation of DNA at 94°C, 30 seconds for annealing of primers at 50°C, and 45 seconds for elongation of newly synthesizing DNA strand at 72°C, and final cycle of extension of DNA for 10 minutes at 72°C. The thermal cycler was set to hold at 4-8 °C after completion of all cycles. For the amplification of circular molecules of begomoviruses, Φ29 DNA polymerase was used on the collected samples of cotton species. In rolling circle amplification, a reaction mixture of 20 µl (100 to 200 ng) of genomic DNA was used as a template, 50 µM random hexamer primers, 2 µl 10X Φ29 DNA polymerase reaction buffer (330 mM Tris-acetate [pH 7.9 at 37 °C], 100 mM magnesium acetate, 660 mM potassium acetate, 1% Tween 20, 10 mM DTT) was prepared and incubated at 94°C for 3 minutes to denatur the double-stranded DNA. The mixture was cooled atroom temperature and mixed with 1 mM dNTPs, 5-7 units of Φ29 DNA polymerase and 0.02 unit of pyrophosphatase (to eliminate inhibitory accumulation of pyrophosphate) and incubated at 30°C for 18 to 20 hours. Φ29 DNA polymerase was inactivated at 65°C for 10 minutes (Nahid *et al*., 2008). Genomic DNA of wild species of cotton was also used in Southern hybridization for the detection of DNA A and betasatellites of begomoviruses. In Southern hybridization, PCR product of begomoviruses and betastellites were probed by Biotin DecaLabel DNA Labeling Kit (Protocol was followed according to the instructions of Fermentas).

RESULTS AND DISCUSSION

Available techniques like PCR, Southern hybridation and rolling circule amplification (RCA) were used in the present study for the detection of viral molecules in wild relative of *Gossypium*. The PCR and Southern hybridization revealed that viral molecules of CLCuD were found to be absent in G. *arboreum* and *G. herbaceum* (Table 1). The PCR results showed the presence of begomovirus and betasatellite molecules in species belonging to D genome (*G. thurberii*, *G. herkensii*, and *G. aridum*) and in species belong to AD genome including *G. hirsutum*, *G. barbadense*, *G. mustelinum* and *G. darwanii*. In addition to PCR and Southern hybridization, effort were also made for the detection of circular molecules of begomoviruses by RCA technique, which were confirmed by restriction with unique restriction enzyme i.e. *Kpn*1and *Xho*1. The restriction analysis showed the absence of begomovirus and betasatellites in *G. arboreum* and *G. herbaceum* but viral molecules were found to be present in species belonging to D genome including *G. thurberii*, *G. herkensii* and *G. aridum* (Table 1). Due to the presence of begomoviruses, plants of *G. thurberii* showed typical symptoms of cotton leaf curl disease (CLCuD) including leaf curling, vein clearing and vein swelling (Fig 1). Interestingly none of the begomoviruses was detected in *G. gossypioides* belongs to D_6 and found to be free from typical symptoms of CLCuD. Both of begomovirus and CLCuMB were detected by Southern hybridization from *G. hirsutum* (AD, America), *G. barbadense* (AD, America), *G. darwanii* (AD, America), *G. mustelinum* (AD, America). Likewise begomovirus and CLCuMB were detected in *G. aridum* (D_4, America) but symptoms of CLCuD were not found, which suggests further investigations.

As expected, *G. herbaceum* and *G. arboreum* belonging to A_1 and A_2 genome were found to be free of begomovirus component and is consistent with observations that these species have no symptoms of CLCuD in field conditions. The tetraploid cotton species including *G. barbadense* (AD_2) (Azhar *et al*., 2012), *G. mustelinum* (AD) and *G. darwanii* (AD) showed the presence of CLCuD. It is speculated that allotetraploid cotton became a host of CLCuD after polyploidization where susceptibility genes were provided by wild species belong to D genome. CLCuMV was not detected by

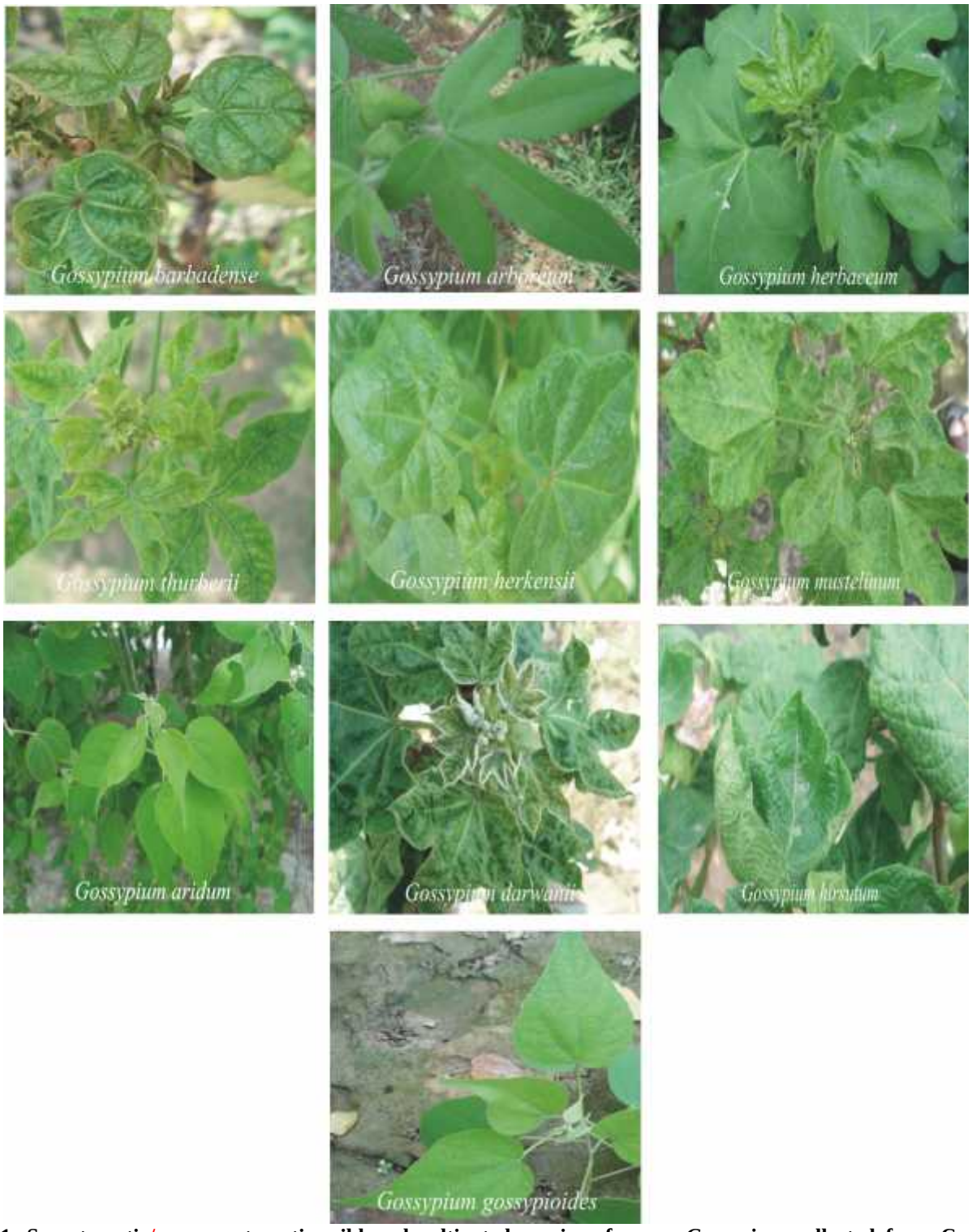

Fig 1. Symptomatic/ non-symtomatic wild and cultivated species of genus *Gossypium* collected from Central Cotton Research Institute (CCRI), Multan, Pakistan.

Southern hybridization and/or RCA in *G. gossypioides* due to low level titer of virus molecule although *G. gossypioides* belongs to D genome (Zhao *et al*., 1998). One possibility of low titer of virus in wild relatives might be the presence of defense mediated by transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS). Because several studies showed that PTGS mechanism are RNA based

host defense system that is able to control the nucleic acid invaders belonging to various nature through *cis*-acting siRNAs (Bartel, 2004, Dunoyer and Voinnet, 2005), reported that PTGS is natural defense mechanism against virus accumulation in the host (Vanitharani *et al*., 2003, Baulcombe, 2004) which was observed in plants for the first time. The function of PTGS has been proved during the development of transgenic resistance against geminiviruses such as African cassava mosaic virus (Vanderschuren *et al*., 2007), mung bean yellow mosaic virus (Pooggin *et al*., 2003), tomato yellow leaf curl virus (Abhary *et al*., 2006, Zrachya *et al*., 2007), Sri Lankan cassava mosaic virus and East African mosaic virus (Chellappan *et al*., 2005).

Earlier investigations showed that this species has been undergone in recombination process with A genome species (Wendel and Cronn, 2002). It is likely that during this hybridization resistance from African genomes (A-, F-, B-, or E-) and D genome species of cotton against begomovirus complexes was transferred in *G. gossypioides* (Cronn *et al*., 2003). One of the challenges to cotton breeders is that how *G. arboreum* could be used in interspecific crosses for the improvement of existing varieties of *G. hirsutum*? Whereas synthetic allotetraploids also showed susceptibility to CLCuD under field conditions (Ahmad *et al*., 2011). We suggest two possible approaches; one involving crosses between *G. arboreum* and *G. gossypioides* followed by chromosome doubling to yield allotetraploid cotton and then backcrosses with *G. hirsutum*. The other approach is the improvements in yield and fiber quality characteristics of *G. arboreum* through interspecific crosses termed as "hirsutization of *G. arboreum*". On the other hand, mapping population for resistance in *G. gossypioides* can be generated by crossing with CLCuD susceptible species belong to D genome.

Table 1. Southern hybridization and PCR results in cultivated and wild species of genus *Gossypium* collected from CCRI, Multan, Pakistan

Name of species	Genome	Southern results		Detection of Betasatellite By PCR	Presence or absence of symptoms of CLCuD
		DNA-A	Betasatellite		
G. herbaceum	A_1	-	-	-	-
G. arboreum	A_2	-	-	-	-
G. thurberii	D_1	+	-	+	+
G. herkensii	D_{2-2}	+	+	+	+
G. aridum	D_4	+	+	+	-
G. gossypioides	D_6	-	-	-	-
G. hirsutum	AD_1	+	+	+	+
G. barbadense	AD_2	+	+	+	+
G. mustelinum	AD	+	-	+	+
G. darwanii	AD	+	+	+	+

Acknowledgements: MTA was supported by a PhD grant from Higher Education Commission (HEC), Islamabad (Pakistan). We are thankful to Director of CCRI for provision of fresh leaf tissue of wild cotton species. The authors are grateful for the support of NIBGE in conducting this study.

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