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DEVELOPMENT AND CHARACTERIZATION OF AN INTERSPECIFIC *Gossypium hirsutum* x *Gossypium armourianum* HYBRID

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ABSTRACT

Interspecific hybrid between *Gossypium hirsutum* (AADD) cv. F 1861 and *G. armourianum* (DD) Acc. PAU 1 was generated and characterized along with parents at morphological and molecular levels. Female parent (F 1861) had erect growth habit, cream petals, palmate leaves, green stem, cream anthers, hairy stem and leaves; whereas male parent (PAU 1) had spreading growth habit, yellow petals, cordate leaves, reddish green stem, yellow anthers and glabrous plant body. The growth habit, petal colour, leaf shape and size of interspecific F_1 hybrid were intermediate. Plant stem colour and hairiness, leaf pubescence and anther colour of *G. armourianum* Acc. PAU 1 were observed to be dominant as hybrid fully resembled Acc. PAU 1 for these characters. Petal spot was absent in F 1861 and present in PAU 1as well as hybrid. Variable expression of petal spot, anther colour and filament colour was observed in F_1. The interspecific F_1 hybrid was male-sterile. Significant differences were observed between the pollen size of parents as well as parents and their hybrid. Of the 33 SSR markers polymorphic between the parents, 8 markers unambiguously confirmed the hybrid status of interspecific hybrid. This hybrid may serve as a useful genetic resource for the transfer of cotton leaf curl disease resistance to American cotton.

Key words: American cotton, cotton leaf curl disease resistance, epigenetics, hybridization, SSR markers, wild *Gossypium*

INTRODUCTION

Cotton (genus *Gossypium*) belongs to the family *Malvaceae* and is a highly diverse genus. This genus comprises of approximately 50 species, including 45 diploids (2n = 2x = 26) and five allotetraploids (2n = 4x = 52) (Fryxell *et al*., 1992). Of these, 4 species are cultivated amongst which 2 (*G. hirsutum* and *G. barbadense*) are tetraploid while 2 (*G. arboreum* and *G. herbaceum*) diploids. *G. hirsutum*, also known as American cotton, is the most widely grown cotton species world-wide. *G. armourianum*, one of 13 wild diploid D-genome species, reportedly possesses resistance to certain diseases and pests including pink bollworm (Brazzel *et al*., 1956) and jassid (Narayanan *et al*., 1984; Pushpam and Raveendran, 2006). Many examples highlighting the role of wild and cultivated *Gossypium* diploid species as sources of genes of economic importance are available (Mehetre *et al*., 2002; Kebede *et al*., 2007). Recently Nazeer *et al*. (2014) have reported the recovery of interspecific derivatives between *G. hirsutum* and *G. arboreum* possessing high tolerance to cotton leaf curl disease (CLCuD) – a dreaded disease of American cotton in Pakistan

and Indian states of Punjab, Haryana and Rajasthan. *G. armourianum* Acc. PAU 1 possesses resistance to CLCuD under field conditions. Therefore, interspecific crosses between *G. hirsutum* and *G. armourianum* were attempted to develop F_1 hybrid and subsequently back cross derivatives to introgress useful traits especially CLCuD resistance in American cotton.

MATERIALS AND METHODS

The interspecific hybrid between *G. hirsutum* cv. F 1861 x *G. armourianum* Acc. PAU 1 was developed during 2012 using F 1861 as the female parent. F 1861 is an American cotton variety developed by PAU, Ludhiana, whereas *G. armourianum* Acc. PAU 1 was procured from ICAR-Central Institute for Cotton Research (CICR), Regional Station, Coimbatore (Tamil Nadu). The parental line F 1861 was planted in field in April 2013 and *G. armourianum* Acc. PAU 1 is being maintained as perennial. No fertilization barriers between these *Gossypium* species were encountered in this direction and the F_1 seeds were obtained with ease through direct crossing in field at PAU Regional Station, Faridkot during *kharif* 2012. One day prior to anthesis at the candle stage, F 1861 was emasculated by removing anthers. The emasculated stigma was covered with a small straw pipe to prevent contamination by undesirable pollen grains. One day prior to pollination, flowers of male parent *G. armourianum* Acc. PAU 1 were tied at pedicel whereas, the other end of thread was tied at the one-third distance from tip of flower to ensure the purity of pollen. The emasculated buds were pollinated the next morning and crossed buds again covered with straw pipe. These crossed flowers were then tagged with date of pollination and parentage written on jewel tags.

F_1 hybrid along with parents was used for recording data on various morphological parameters and molecular analysis. The morphological characters studied included plant growth habit, stem colour and hairiness, leaf shape, leaf lobe number, leaf size (maximum width), leaf pubescence, leaf nectarines, bracts shape, number of serrations on bracts, petal number, petal colour, petal spotting, filament colouration, anther colour, average anther number, pollen fertility, average pollen size and boll shape. All the characters were recorded from fully matured plants. Pubescense on leaves and stem was recorded as presence or absence. Number of nectarines present at midrib on the back side of leaves were counted. Number of anthers was counted from 25 flowers of each parental line and F_1 hybrid, and averaged. Fourth fully matured and expanded leaves from the top of branch were taken and their maximum width was recorded with the help of a measurement scale. Twenty five leaves in each parent and F_1 hybrid were taken and average leaf size noted. To analyse pollen fertility, flowers were collected in morning on the day of anthesis between 10 am to 11 am. In the evening one day prior to collection, flowers of lines to be used for fertility test were tied at pedicel with a thread from one end, whereas the other end of thread was tied to ensure purity of pollen. Next day after collection, pollen fertility was recorded by dusting pollen in 2.5% acetocarmine dye for 30 min. under a compound microscope. Only large, bright and red grains were considered fertile. The total number of pollen grains examined to assess pollen fertility were 1220 in F 1861, 1308 in *G. armourianum* Acc. PAU 1 and 1235 in interspecific F_1 hybrid. Micrometry technique was used to measure the size of pollen grains under a microscope for about 100 pollens in each parent and F_1 hybrid. Z-test analysis was performed to test the significance in average pollen sizes and fertility (Sukhatme and Panse, 1995) . Similarly, data on leaf size and anther number for each parent and hybrid were subjected to Student's t-test to analyze the differences among them.

A set of 55 cotton specific simple sequence repeat (SSR) primer pairs were used to document polymorphism between parents and a subset of polymorphic markers was employed for hybridity confirmation of interspecific F_1 hybrid. Total genomic DNA from parents and interspecific F_1

hybrid was extracted using NucleoSpin® Plant II DNA purification kit (Macherey-Nagel, Germany). The quality and quantity of DNA was assessed using Nano-Drop spectrophotometer (Thermo Scientific NanoDrop™ 1000 Spectrophotometer). DNA was amplified using 1X PCR buffer (3.0 µL), 1.5 mM $MgCl_2$ (1.0 µL), 200 µM dNTPs (3.0 µL), 0.5 µM forward (1.0 µL) and reverse (1.0 µL) primers, 1 unit Taq polymerase (0.3 µL), 40 ng DNA template (2.0 µL) and deionised water (3.7 µL) in a 15 µL reaction mixture. After PCR amplification, amplified products were resolved using 6% polyacrylamide gel. The gels were visualized under UV light and photographed using photo gel documentation system (Alphaimager HP, Alpha Innotech).

RESULTS AND DISCUSSION

Comparison of morphological characters of parents *G. hirsutum* cv. F 1861, *G. armourianum* Acc. PAU 1 and their hybrid are presented in Table 1. Interspecific F_1 hybrid manifested either dominance or intermediate expression for various morphological traits. The growth habit (Plate 1), petal colour, leaf shape and size of interspecific hybrid were found to be intermediate.

In F 1861, the average leaf size in female and male parents was 13.8 and 7.2 cm, respectively, whereas in F_1 hybrid it was 3.7 cm with a range of 3.0-4.5 cm which is almost intermediate of the parental average leaf sizes (Table 1). Significant differences were observed between leaf sizes of parents as well as between each parent and hybrid. Leaf shape of *G. hirsutum* cv. F 1861 was palmate with 5 lobes, whereas *G. armourianum* Acc. PAU 1 had cordate leaves. In case of F_1 hybrid, leaves were palmate with 3-4 lobes and were reduced in size as compared to *G. hirsutum* (F 1861) parent leaves. The results are in agreement with Pushpum and Raveendran (2006) wherein they have reported intermediate leaf shape and size in hybrids between *G. hirsutum* and *G. armourianum*. Similar intermediate expression of plant growth habit, leaf size and petal colour have

Table 1: Comparison of morphological characters among parents and F_1 hybrid

Characters	*G. hirsutum* cv. F 1861	*G. armourianum* Acc. PAU 1	F_1 hybrid
Plant growth habit	Erect	Spreading	Erect and semi-spreading
Plant stem coloration	Green	Reddish green	Reddish green
Plant stem hairiness	Present	Absent	Absent
Leaf shape	Palmate	Cordate	Palmate
Leaf lobe (No.)	5	-	3 – 4
Leaf pubescence	Present	Absent	Absent
Nectaries on leaf midribs	Present	Present	Present
Bract type	Cordate with serrations	Caducous bracts	Cordate with serrations
Serrations on bracts (No.)	9 – 11	No bracts	5 – 6
Average leaf size (cm)	13.8 (12.0-15.5)	3.7 (6.2-8.5)	7.2 (3.0-4.5)
Sepals (No.)	5	5	5
Flower petal colour	Cream	Yellow	Creamy yellow
Flower petal spotting	Absent	Present	Variable expression
Flower filament colouration	Colourless	Colourless	Variable expression
Pollen colour	Cream	Yellow	Yellow
Connective colour	Colourless	Coloured	Variable expression
Anthers (No.)	122.7 (103-136)	124.4 (117-135)	117.6 (101-139)
Boll shape	Elliptical with pointed tip	Oval with pointed tip	Oval with pointed tip
Pollen fertility (%)	94.26	96.63	2.19
Pollen size (µm)	116.61 (93.6-132.6)	103.22 (70.2-109.2)	69.52 (31.2-109.2)

Note: Overall range is given in parentheses.

been reported in other interspecific *Gossypium* species hybrids such as between *G. davidsonii* x *G. anomalum* (Mehetre *et al.*, 2003), *G. hirsutum* x *G. arboreum* (Ahmad *et al*., 2011; Tahir *et al*., 2011), *G. herbaceum* x *G. australe* (Liu *et al*., 2015), and *G. arboreum* x *G. thurberi* (Manisha *et al*., 2007). However, some workers have reported dominance for these characters. For example, *G. hirsutum* x *G. raimondii* triploid hybrid resembled the paternal parent in growth habit (Saravanan *et al*., 2007). Results of present study do not correspond to these studies for growth habit, petal colour, and leaf shape. The differential results reiterate that dominance is a concept and not a rule. Plant stem colouration and hairiness (Plate 1), leaf pubescence and anther colour of *G. armourianum* Acc. PAU 1 were found dominant as hybrid fully resembled the male parent for these characters.

Female parent and hybrid had 3 normal bracts with teeth like serrations. Size of bracts was larger in female parent than in hybrid. The number of serrations varied between 9-11 and 5-6 in female parent and hybrid, respectively. An interesting feature observed in wild diploid male parent (*G. armourianum* Acc. PAU 1) was the presence of caducous bracts (bracts shed at very early stage even before the opening of flower). However in hybrid bracts shed before the opening of boll but were present on the flowers (Plate 1). Anther number ranged between 103-136, 117-135 and 101-139 in. F 1861, Acc. PAU 1 and their hybrid, respectively. Average anther number from 20 flowers was 122.7, 124.4 and 117.5 in female parent, male parent and their hybrid, respectively. No significant differences were observed among the anther numbers of parents and hybrid.

Average pollen fertility was recorded to be 94.26, 96.63 and 2.19% in *G. hirsutum* cv. F 1861, *G. armourianum* Acc. PAU 1 and their hybrid, respectively. Significant differences were observed for pollen fertility between parents and hybrid, whereas parents were found to be at par for this trait. Pushpum and Raveendran (2006) reported 9.04% average pollen fertility with a range of 4.1 to 15.0% in *G. hirsutum* x *G. armourianum* hybrids and 9.67% (range: 5.7 to 15.2%) in *G. hirsutum* x *G. raimondii* hybrids produced using 12 different *G. hirsutum* accessions. The differences may be attributed to the different genotypes used in these experiments. Average pollen size in *G. hirsutum* cv. F 1861, *G. armourianum* Acc. PAU 1 and their hybrid was determined to be 116.61, 103.22 and 69.52 µm, respectively. More variation was observed in the pollen grain size of hybrid as compared to parents. Significant differences were observed between the pollen sizes of the parents as well as between parents and their hybrid.

As usual, petal spot was absent in American cotton parent and present in *G. armourianum* Acc. PAU 1 (Plate 1). In the F_1 hybrid, variation for petal spot size and intensity was observed in different flowers of the same plant (Plate 2). It ranged from dark pink colour and full size as that of male parent through reduced intensity and size to complete absence on the same plant of hybrid. However, Ahuja and Dhayal (2007) reported complete dominance of the petal spot in intra *hirsutum* crosses involving wild type x mutant strains. Other workers have reported reduction in the color intensity of petal spot in F_1 hybrids e.g. in case of *G. hirsutum* x *G. arboreum* hybrid (Tahir *et al*., 2011; Ahmad *et al*., 2011). Interspecific hybrids between *G. hirsutum* and *G. barbadense*, cultivated in the country show intermediate expression of petal spot. Similar expression was observed in case of filament colouration in the present study (Plate 3). It was colourless in both female and male parents. But in case of F_1 hybrid, filament was either colourless or coloured in different flowers and, even few colourless and few coloured filaments were observed in the same flower. Connective, the portion that connects the filament and the anther, was colourless in female parent and coloured in male; whereas in hybrid both colourless and coloured connectives were in the same flower and one of the either in different flowers on the same plant (Plate 3). In the present investigation, variable expression of petal spot, filament colouration, and colour of connective in *G. hirsutum* x *G. armourianum* hybrid might be due to epigenetics. Epigenetics refers to heritable gene expression patterns determined by how the DNA of a gene is packaged rather than its primary DNA sequence involving interaction of a wide variety of protein complexes and regulatory mechanisms (Bender, 2002). Rapp and Wendel (2005) viewed 'epigenetics' as the alteration of phenotype, morphological or molecular, without change in either the coding sequence of a gene or the upstream promoter region. Nascent allopolyploids are reported to be associated with variation and instability

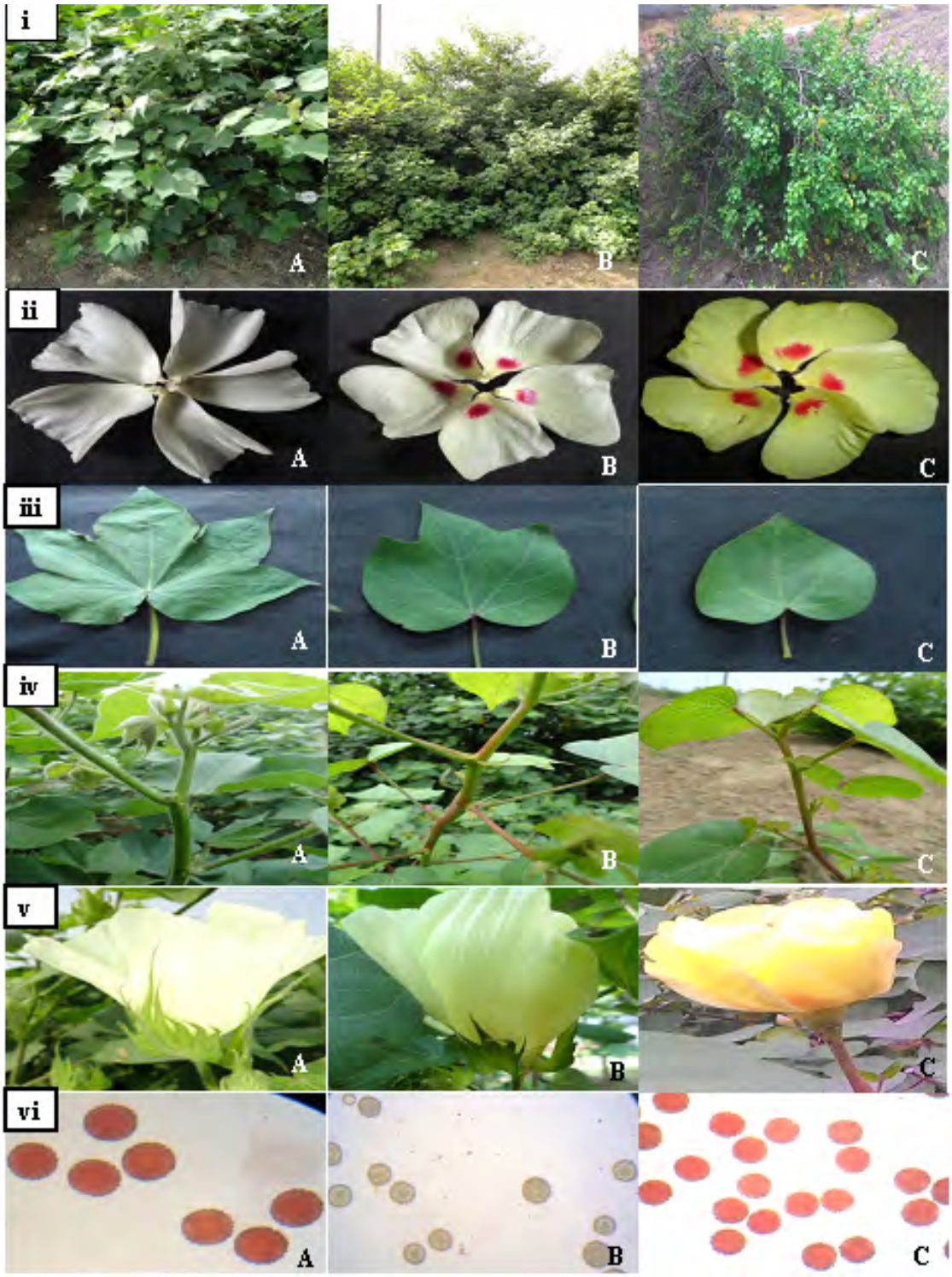

Plate 1: Comparison of i) plant growth habit, ii) petal number and colour, iii) leaf shape and number of lobes, iv) stem colour and hairiness, v) presence/absence of bracts, and vi) pollen fertility and pollen size. Panel A: *G. hirsutum* cv. F 1861, Panel B: interspecific F_1 hybrid, and Panel C: *G. armourianum* Acc. PAU 1

in phenotypes that cannot be accounted for by conventional Mendelian transmission genetics or chromosomal aberrations (Comai, 2000; Comai *et al*., 2000). The affected traits are diverse, including timing of flowering, overall plant habit, leaf morphology, and homeotic transformations

Plate 2: Variation in expression of size and intensity of petal spot in interspecific *G. hirsutum* x *G. armourianum* hybrid

in floral morphology (Comai *et al*., 2000). Various causes, including increased variation in dosage-regulated gene expression, altered regulatory interactions, and rapid genetic and epigenetic changes, which are probably conferred by genome-wide interactions (reviewed in Osborn *et al*., 2003) has been suggested for such altered expressions.

For hybridity confirmation, parents viz. *G. hirsutum* cv. F 1861 and *G. armourianum* Acc. PAU 1 were subjected to polymorphic analysis employing 55 cotton specific SSR markers. Thirty three markers were observed to be polymorphic between parents, thus registering 64% polymorphism, whereas 18 markers showed monomorphic amplification pattern and four primer pairs (NAU2274, NAU2896, NAU3811, and NAU3384) did not amplify. Of the 33 polymorphic primers, SSR markers namely BNL3590, BNL3917, BNL3948, NAU1070, NAU2156, NAU3561, NAU3775 and NAU5345 were selected to confirm the hybrid status of interspecific hybrid as polymorphic bands from both the parents were present in the hybrid. None of the other polymorphic primers was selected as it would have revealed same banding pattern in F_1 hybrid and the selfed plant as null allele was observed in male parent with respect to the female parent band. Perusal of Plate 4 revealed that BNL3948 amplified one locus in *G. hirsutum* cv. F 1861 and one in *G. armourianum* Acc. PAU 1. In the interspecific hybrid, both the loci corresponding to the parental species were amplified. BNL3590 amplified one locus in female parent and three loci in male parent; all the four loci were amplified in the F_1hybrid. NAU2156 amplified three loci in female parent and two loci in male parent one being monomorphic between the parents, all the four loci were amplified in the hybrid. NAU5345 amplified one locus in female parent and three loci in male

Plate 3: Filament and anther colour variation in A) *G. hirsutum* cv. F1861, B) variable expression in interspecific F_1 hybrid, and C) *G. armourianum* Acc. PAU 1

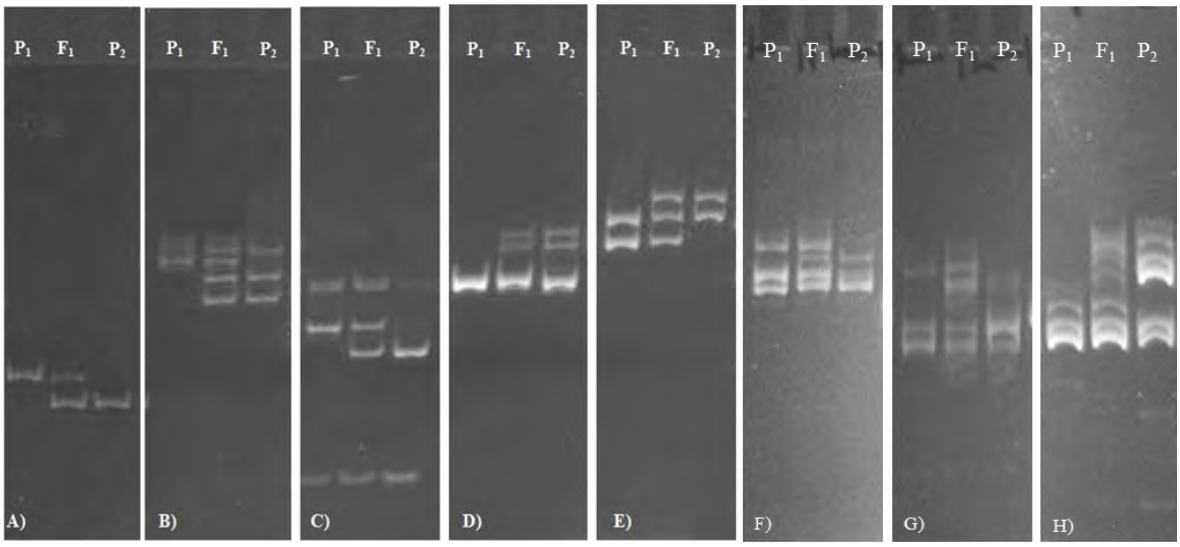

Plate 4: Amplification pattern of SSR primers (A) BNL3948, (B) BNL3590, (C) NAU2156, (D) NAU5345, (E) NAU3775, (F) NAU3917, (G) NAU1070 and (H) NAU3561in *G. hirsutum* cv. F 1861 (P_1), *G. armourianum* Acc. PAU 1 (P_2) and their hybrid (F_1)

parent; one locus corresponding to the female parent was monomorphic with male parent and therefore three loci were amplified in hybrid, thus confirming its hybridity. Similarly, NAU1070, NAU3561, NAU3775 and NAU3917 unambiguously confirmed the hybrid status of the F_1 as shown in Plate 4. Two polymorphic loci amplified by NAU3775, four by NAU3561, two by each NAU1070 and NAU3917 in F_1 hybrid confirmed its hybridity.

Several reports of hybridity confirmation of interspecific and intraspecific cotton hybrids using molecular markers are available. For example, Dongre and Parkhi (2005) and Dongre *et al*. (2011) used combination of RAPD, ISSR, and SSR markers for identification of intra *hirsutum* (H6) and interspecific *G. hirsutum* x *G. barbadense* (Phule-388) hybrids, respectively. In other recent studies, hybridity confirmation of interspecific hybrids between *G. arboreum* and *G. hirsutum* using SSR markers has been reported by Virk (2014) and Vij *et al*. (2016). Similarly, Zhang *et al*. (2014) confirmed the hybrid status of an interspecific hexaploid hybrid between *G. hirsutum* x *G. anomalum* employing microsatellite markers.

The reported interspecific hybrid between *G. hirsutum* cv. F 1861 x *G. armourianum* Acc. PAU 1 is an important genetic resource for the cotton breeders. It has been observed to be resistant to cotton leaf curl disease under field conditions. CLCuD is the major disease of American cotton in North Indian cotton growing states and Pakistan and results in heavy losses in seed cotton yield especially if it appears at seedling stage. All the CLCuD resistant *G. hirsutum* cultivars/stocks have become susceptible due to the appearance of recombinant virus strains. Efforts are being made to use this hybrid for the transfer of CLCuD resistance through crosses with the susceptible American cotton lines. This interspecific hybrid (ADD) not only provides an opportunity to study chromosomal behaviour during meiosis but also investigating the genic and genomic consequences of allopolyploidy.

REFERENCES

Abdel-Mawgood, A.L., Ahmed, M.M.M. and Ali, B.A. 2006. Application of molecular markers for hybrid maize (*Zea mays* L.) identification. *Journal of Food Agriculture and Environment*, **4**: 176-178.

Adams, K.L., Percifield, R. and Wendel, J.F. 2004. Organ-specific silencing of duplicated genes in a newly synthesized cotton allotetraploid. *Genetics*, **168**: 2217-2226.

Ahmad, S., Mahmood, K., Hanif, M., Nazeer, W., Malik, W., Qayyum, A., Hanif, K., Mahmood, A. and Islam, N. 2011. Introgression of cotton leaf curl virus resistance genes from asiatic cotton (*Gosssypium arboreum*) into upland cotton (*G. hirsutum*). *Genetics and Molecular Research*, **10**: 2404-2414.

Ahuja, S.L. and Dhayal, L.S. 2007. Comparative characteristics and gene action in three petal-spotted mutants of *Gossypium hirsutum*. *Journal of Genetics*, **86**: 81-84.

Altman, D.W., Stelly, D.M. and Kohel, R.J. 1987. Introgression of the glanded-plant and glandless-seed trait from *Gossypium sturtianum* Willis into cultivated upland cotton using ovule culture. *Crop Science*, **27**: 880-884.

Arya, L., Verma, M. and Lakhanpaul, S. 2014. Diagnostic set of microsatellite markers for hybrid purity testing and molecular identification of hybrids and parental lines in sorghum. *Journal of Plant Science and Research*, **1**: 103-107.

Beasley, C.A. and Ting, I.P. 1973. The effects of plant growth substances on *in vitro* fiber development from fertilized cotton ovules. *American Journal of Botany*, **60**: 130-139.

Bender, J. 2002. Plant epigenetics. *Current Biology*, **12**: R412-14.

Brazzel, J.R. and Martin, D.F. 1956. *Resistance of Cotton to Pink Bollworm Damage*: Tech Bull, Texas Agricultural Research Station, Texas, USA.

Comai, L., Tyagi, A.P., Winter, K., Holmes-Davis, R., Reynolds, S.H., Stevens, Y. and Byers, B. 2000. Phenotypic instability and rapid gene silencing in newly formed *Arabidopsis* allotetraploids. *Plant Cell*, **12**: 1551-1567.

Comai, L. 2000. Genetic and epigenetic interactions in allopolyploid plants. *Plant Molecular Biology*, **43**: 387-399.

Dongre, A. and Parkhi, V. 2005. Identification of cotton hybrid through the combination of PCR based RAPD, ISSR and microsatellite markers. *Journal of Plant Biochemistry and Biotechnology*, **14**: 53-55.

Dongre, A.B., Raut, M.P., Bhandarkar, M.R. and Meshram, K.J. 2011. Identification and genetic purity testing of cotton F_1 hybrid using molecular markers. *Indian Journal of Biotechnology*, **10**: 301-306.

Fryxell, P.A., Craven, L.A. and Stewart, J.M.D. 1992. A revision of *Gossypium* section *Grandicalyx* (Malvaceae), including the description of six new species. *Systematic Botany*, **17**: 91-114.

Kebede, H., Burow, G., Dani. R.G. and Allen, D. 2007. A-genome cotton as source of genetic variability for upland cotton (*Gossypium hirsutum*). *Genetic Resources and Crop Evolution*, **54**: 885-895.

Liu, Q., Chen, Y., Chen, Y., Waang, W., Chen, J., Zhang, T. and Zhou, B. 2015. A new synthetic allotetraploid ($A_1A_1G_1G_1$) between *Gossypium herbaceum* and *Gossypium australe*: Bridging for simultaneously transferring favourable genes from these two diploid species into upland cotton. *PLOS ONE*, doi: 10.1371/journal.pone.0123209.

Mangibas, N.L. and Villegas, C.V. 2004. Microsatellite markers in hybridity test to identify true hybrids of sugarcane. *Philippines Journal of Crop Science*, **29**: 23-32.

Manisha, K., Mehetre, S.S., Gahukar, S., Shinde, G. and Patil, V. 2007. Cyto-morphological and RAPD analysis of F_1, F_2 and BC_1 generations of a cross *Gossypium arboreum* x *Gossypium thurberi*. *Cytologia*, **60**: 379-388.

Mehetre, S.S., Patil, S.C., Pawar, S.V., Pardedhi, S.U., Shinde, G.C. and Aher, A.R. 2004. Ovulo embryo cultured hybrids between amphidiploids (*G. arboreum* x *G. anomalum*) and *G. hirsutum*. *Current Science*, **87**: 286-289.

Mehetre, S.S., Patil, S.D. and Gawande, V.L. 2002. Introgression of disease and pest resistance from wild to cultivated species of *Gossypium* – A review. *Journal of Cotton Research and Development*, **16**: 178-181.

Mohan, C., Shanmugasundaram, P. and Senthil, N. 2013. Identification of true hybrid progenies in cassava using simple sequence repeat (SSR) markers. *Bangladesh Journal ofBotany*, **42**: 155-159.

Nandakumar, N., Singh, A.K., Sharma, R.K., Mohapatra, T., Prabhu, K.V. and Zaman, F.U. 2004. Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. *Euphytica,* **136**: 257-264.

Narayanan, S.S., Singh, J. and Verma, P.K. 1984. Introgressive gene transfer in *Gossypium* – Goals, problems, strategies and achievements. *Cotton et FibresTropicales*, **19**: 10-24.

Nazeer, W., Tipu, A.L., Ahmad, S., Mahmood, K., Mahmood, A. and Zhou, B. 2014. 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*. *PLOS ONE* doi: 10.1371/journal. pone.0111861.

Osborn, T.C., Piers, J.C., Birchler, J.A., Auger, D.L., Chen, Z.J., Lee, H.S., Comai, S., Madlung, A., Deorge, R.W., Colot, V. and Martienssen, R.A. 2003. Understanding mechanisms of novel gene expression in polyploids. *Trends in Genetics***, 19**: 141-147.

Pallavi, H.M., Gowda, R., Shadakshari, Y.G., Bhanuprakash, K. and Vishwanath, K. 2011. Identification of SSR markers for hybridity and seed genetic purity testing in sunflower (*Helianthus annuus*). *Helia,* **34**: 59-66.

Pushpam, R. and Raveendran, T.S. 2006. Production of interspecific hybrids between *Gossypium hirsutum* and jassid resistant wild species *G. raimondii* and *G. armourianum*. *Cytologia,* **71**: 407-418.

Rapp, R.A. and Wendel, J.F. 2005. Epigenetics and plant evolution. *New Phytologist*, **168**: 81-91.

Reddy, M., Hunje, R., Biradar, D.P. and Vyakarnahal, B.S. 2007. Characterization of cotton hybrids and parental lines using morphological characters. *Karnataka Journal of Agricultural Sciences*, **20**: 511-513.

Saravanan, N.A., Ram, S.G., Thiruvengadam, G., Ravikesavan, R. and Raveendran, T.S. 2007. Production and fertility restoration of an interspecific hybrid between *Gossypium hirsutum* L. and *G. raimondii* U. *Cytologia,* **72**: 195-203.

Solanki, R.K., Singh, S. and Kumar, J. 2010. Molecular marker assisted testing of hybridity of F_1plants in lentil. *Journal of Food Legumes***, 23**: 21-24.

Subashini, V., Shanmugapriya, A. and Yasodha, R. 2014. Hybrid purity assessment in eucalyptus F_1 hybrids using microsatellite markers. *Biotechnology,* **4**: 367-373.

Sukhamte, P.V. and Panse, V.G. 1995. *Statistical Methods for Agricultural Workers*. ICAR, New Delhi. India.

Tahir, M.S., Khan, N.U.I. and Rehman, S.U. 2011. Development of an interspecific hybrid (triploid) by crossing *Gossypium hirsutum* and *G. arboreum*. *Cytologia*, **76**:193-199.

Tamilkumar, P., Jerlin, R., Ganesan, K.N., Jeevan, R.J. and Raveendran, M. 2009. Fingerprinting of rice hybrids and their parental lines using microsatellite markers and their utilization in genetic purity assessment of hybrid rice. *Research Journal of Seed Science,* **2**: 40-47.

Vij, S., Pathak, D., Kaur, N., Pahwa, K. and Gill, M.S. 2016. Development and molecular confirmation of interspecific hybrids between *Gossypium hirsutum* and *G. arboreum*. *Agricultural Research Journal*, (accepted for publication).

Virk, J.K. 2014. *Development and Characterization of Gossypium hirsutum x Gossypium arboreum Hybrids*. Ph.D. thesis. Punjab Agricultural University, Ludhiana, Punjab, India.

Zhang, X., Zhai, C., He, L., Guo, Q., Zhang, X., Xu, P., Su, H., Gong, Y., Ni, W. and Shen, X. 2014. Morphological, cytological and molecular analyses of a synthetic hexaploid derived from an interspecific hybrid between *Gossypium hirsutum* and *Gossypium anomalum. Crop Journal*, **2**: 272-277.