Introgression of Pigment Gland Morphogenesis Delay into Upland Cotton: Potential of DNA Markers to Monitor Parental Contribution to Progenies

I. Vroh Bi, G. Mergeai, J.P. Baudoin, J.M. Jacquemard, P. du Jardin

1Unité de Phytotechnique des Régions Intertropicales, Faculté Universitaire des Sciences Agronomiques, 2, Passage des déportés, B-5030 Gembloux, Belgium
2Station d'Amélioration des Plantes, Centre de Recherches Agronomiques, 4, rue du Bordia, 5030 Gembloux
3Unité de Biologie Végétale, Faculté Universitaire des Sciences Agronomiques, 2, Passage des déportés, B-5030 Gembloux, Belgium

ABSTRACT

The delay of pigment gland morphogenesis in the seed confers to several Australian wild diploid cottons the glandless-seed/glanded-plant trait. To introgress this trait from G. sturtianum Willis (2n = 2x = 26, 2CI genome) into G. hirsutum L. (2n = 4x = 52, 2AD genome), we used bridge crosses to synthesize two trispecies hybrids, G. hirsutum-G. raimondii Ulbrich - G. sturtianum (HRS) and G. thurberi Torado - G. sturtianum - G. hirsutum (TSH). Recurrent backcrossing of these hybrids to G. hirsutum produced progenies expressing the desired trait at different levels. The objective of this study was to assess the genomic contribution of the parental species to their progenies with random amplification polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) probes. The use of 50 decamer primers yielded 375 bands of which 339 were polymorphic between parents. Among 45 bands specific to the donor G. sturtianum, 20 and 18 that segregated in backcrosses, were observed in HRS and TSH, respectively. The American wild diploid species used as bridge showed 12 and 13 specific bands for G. raimondii (2m = 2x = 26, 2DI genome) and G. thurberi (2n = 2x = 26, 2DI genome), respectively. Genetic distances between G. hirsutum and the wild species involved in the cross were determined from RAPD data. This study allowed choice within backcross progenies that shared the highest similarity to the cultivated cotton. The parental origin of chromosomes in the trispecies hybrids and the backcrosses were then identified using RFLP probes specific to cotton chromosomes. The results are discussed in relation to the expression of the desired trait.

Introduction

Gossypium contains about 50 diploid and tetraploid species. The diploid species (2n = 2x = 26) fall into 8 different cytotypes designated A, B, C, D, E, F, G and K (Fryxell, 1979; Endrizzi et al., 1985; Gorham et al., 1996). Cotton is not only the leading fiber crop, it is also the second best potential source of plant proteins, and the fifth best oil-producing plant (Textor, 1993). One of the main traits delineating the Gossypium genus is the presence of pigment glands throughout the plant. Pigment glands (also called gossypol glands), contain polyphenolic compounds which confer insect resistance to cotton plant. However, the presence of glands in cottonseed is undesirable because gossypol and derivatives are toxic to man and other monogastric animals. Glanded cottonseed kernel containing from 0.6 to 2.0% gossypol has limited nutritional uses (Lusas and Jividen, 1987), and completely glandless cotton (McMichael, 1954) is susceptible to several insect pests. The objective of our research is to develop an upland cotton G. hirsutum L. (2n = 4x = 52, 2AD genome), having glandless or low-gossypol seed for feed and food uses, and a high level of gossypol in the remaining organs to resist pests. Using the Australian wild diploid species G. sturtianum Willis (2CI genome) as donor, and an American wild diploid species (G. raimondii Ulbr. 2DI genome, or G. thurberi Tod.; 2DI genome) as bridge, trispecies hybrids and backcross progenies were obtained through recurrent selection.

In the past few years, new strategies based on marker-assisted selection have been proposed to reduce time and effort in developing new varieties (Rafalski and Tingey, 1983; Young and Tankolye, 1988; Lee, 1998). Molecular markers are very efficient tools to monitor alien DNA introgression during breeding programs. In the present study, the trispecies hybrids and their backcross progenies (BC) were evaluated to test the suitability of RAPDs and RFLPs for detecting introgression, and improving selection efficiency.

Materials and methods

Plant materials. Two trispecies hybrids (G. thurberi-G. raimondii-G. hirsutum (TSH), and G. hirsutum -
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G. *raimondii* - *G. sturtianum* (HRS) were involved in recurrent backcrosses with *G. hirsutum* to produce BC<sub>1</sub>, BC<sub>2</sub>, BC<sub>3</sub> and BC<sub>3</sub>sp (BC: selfed progenies), thanks to *in vitro* culture of seed embryos, application of growth regulators after manual pollinations, and grafting of unbalanced progenies on vigorous *G. hirsutum* plantlets.

**DNA extraction.** An important step in DNA marker-assisted selection is the efficient isolation of plant DNA. We used activated charcoal in a modified CTAB method to obtain a simple but efficient DNA extraction procedure (Vreb Bi et al., 1996).

**Molecular analysis.** RAPD reactions and statistic analyses were performed according to Vreb Bi et al., 1997 and Mergeai et al., 1998. In addition, the robustness of similarity estimates was analyzed by the bootstrap technique (Felsenstein, 1986), using the geneic distance of Nei (Nei and Lei 1979). Restriction endonuclease digestion, Southern blotting, labelling and hybridization of RFLP probes were performed as described in Reinsch et al. (1994), with slight modifications (7 µg of genomic DNA/reaction. 50 µg of PCR-amplified probe-DNA, 10 ml hybridisation mix, Kodak XOMAT AR film, one week-exposure minimum). Forty-nine RFLP markers of known map positions (Reinsch et al., 1994) were tested on genomic DNA digested with six restriction enzymes (BamH1, CfoI, EcoR1, EcoR3, Hind3, XbaI). These clones were kindly provided by M11 insert Professor A.H. Paterson of Texas A&M University (USA).

**Results and discussion**

**RAPD analysis.** Seventy five decamer primers were screened on parental species, trispecies hybrids and 27 BC<sub>1</sub> plants. Thirty primers showing consistently reproducible bands were used for further studies. The analysis of species-specific bands confirmed the triparental origin of both hybrids TSH and HRS. Owing to the dominant nature of RAPD markers, only markers specific to the wild parents (bands present in the wild parents and not in the cultivated cotton) could be used to detect introgression. An example of introgression and segregation of such specific RAPD bands in the trispecies hybrid HRS and its BC<sub>1</sub> is summarized in Table 1. The thirty primers detected 12, 13, and 49 specific bands from G. *raimondii*, G. *thurberi* and G. *sturtianum* (the donor parent), respectively. Of the 49 G. *sturtianum*-specific bands, 22 were present in the trispecies hybrids and 17 segregated in the BC<sub>1</sub> progenies (Mergai et al., 1998). Markers that are not transmitted to progenies at each cycle of cross are either located on chromosomes that are not transmitted during meiosis, or constitute markers underlying recombinations that can modify the primer binding sites. Few specific markers of G. *sturtianum* were systematically present in all the BC<sub>1</sub> analyzed. Such bands should be located on C genome chromosomes that are preferentially transmitted to the BC<sub>1</sub> due to their higher pairing affinity with the A and D chromosomes of the other parents, or represent repeated DNA dispersed throughout the genome of G. *sturtianum*.

The reliability of our RAPD data to establish genetic distances within cotton germplasm was checked by studying relationships between the parental species, using UPGMA (unweighted pair group method with arithmetical mean) and Jaccard’s distances (Figure 1). The pattern obtained is in agreement with the current phylogenetic classification of *Gossypium* species based on morphological and cytogenetical studies (Fryxell, 1979; Endrizzi et al., 1985). Taking the most remote species, G. *sturtianum* as outgroup, the robustness of the remaining nodes was assessed on 1000 bootstrap runs by the neighbour-joining method of PHYLIP (Felsenstein 1993). Results are shown on Figure 2. All the four varieties of *G. hirsutum* are tightly clustered together (bootstrap values of 840 to 992), showing that such studies can also reliably group cotton varieties. Analysis of similarity showed that both trispecies hybrids were closer to cultivated cotton than to wild diploids. This is certainly due to the tetraploid nature of the cultivated parent that contributed twice to the hybrids composition, compared to the contribution of each wild diploid species. We used these data to generate Jaccard’s coefficients of similarity between genotypes. Analysis of all crosses revealed 30.6 to 39.4% similarity between G. *hirsutum* and the wild species and 74.3 to 89.0% between the four cultivated varieties, while similarity between BC progenies and cultivated parent varied from 57.7 to 67.2%. This facilitated the choice of BC plant sharing the highest similarity with cultivated cotton.

**RFLP analysis.** Of the 49 RFLP markers amplified by PCR, 41 having specific amplification were labelled and used as probes. Twenty five probes distributed across chromosomes 1, 6, 10, 14, 15, 17, 20, 22, 23 and linkage groups A02, A07, D01, D03, D04, D07, U01, U07 hybridized successfully and generated 106 RFLPs of which, 54 (50.90%) were polymorphic. Analysis of introgression from the donor parent (G. *sturtianum*) showed the presence of 11 and 7 chromosomal segments in the trispecies hybrids HRS and TSH respectively. Introgression of these chromosomal segments was traced in subsequent backcross generations. In the BC<sub>1</sub> expressing the "glandless-seed and glanded-plant" trait, the presence of segments from chromosomes 1, 10, and linkage groups A07 and U07 of G. *sturtianum* was evidenced. This plant contains also a segment of chromosome 1 of G. *thurberi*. Introgression from both bridge species G. *raimondii* and G. *thurberi* was also identified in hybrids and backcrosses, but introgression seemed to be most common from G.
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_rationem_ than from _G. thurberi_. Among all the linkage groups analyzed, the chromosome 1 is the most introgressed with four markers in one or another backcross. In addition to these four markers, the introgression of wild diploid specific markers located on chromosome 15 indicated probable introgression of chromosome 1 segments. Since chromosome 15 is homoeologous to chromosome 1 (Reinsch et al., 1984), one of such chromosome 1 markers was introgressed from _G. sturtianum_ and _G. thurberi_ respectively.

The introgression from wild species was of two types. In the most common type, RFLP alleles characteristic of cultivated and wild cottons were present, and the plants were heterozygous at the introgressed loci. In the second type, one of the _G. hirsutum_ RFLP allele was replaced by the corresponding _G. sturtianum_ or _G. raimondii_ allele. This last type evidenced on chromosome 1 with the probes AI204 and AI593, is consistent with reciprocal recombinations due to probably to multivalent configurations observed at metaphase I (Voh Bi et al., 1998). Evidence of reciprocal recombinations also indicates that the crossing schemes developed in this study can lead to homoeologous chromosomes pairing and intergenomic exchanges.

**Conclusion**

The interest of molecular markers in breeding programs to tag introgression from parents has been demonstrated in many plants (Tank and Hewitt, 1988; Lee 1988). First, we have evaluated RAPD markers as tools for determining relationships between species, varieties, hybrids and backcross progenies of cotton. The results showed that RAPD can be used to differentiate cultivated genotypes of cotton, but also to estimate the genetic contribution of each parent to each member of a segregating population (i.e. backcross). Introgressed individuals whose genome composition most resembles the cultivated cotton genome can be selected for the next cross through genetic distance estimations. This could potentially accelerate the introgression of traits from genetically distant parents like those used in the present program. Second, using markers selected from the developing RFLP map of cotton (Reinsch et al., 1994), we have demonstrated the ability to follow introgression of specific chromosome regions from parents to descendants through multiple generations. The fact that cotton RFLP markers initially developed from the cross _G. hirsutum_ x _G. barbadense_ (Reinsch et al., 1994) works also in wide crosses involving other species shows that this map will prove to be useful in a wide range of cotton breeding programs assisted by DNA markers. Although the introgression of the 'glandless-seed and glanded-plant' trait from Australian wild diploid cottons was previously attempted by cotton breeders (Dilday 1985; Shuijing and Billig 1993), chromosomes acting for the variable expression of this trait in introgressed genetic backgrounds remain unknown. Since the BC progenies analyzed here are segregating for both RFLP markers of known chromosomal positions and the desired trait, the present study is an important step towards the mapping of the 'low-gossypol seed and high-gossypol plant' trait. Indeed, using more RFLP markers in large segregating populations could establish association between chromosomal segments or loci of _G. sturtianum_ and the gland levels in different organs of the plants.

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