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DEVELOPMENT OF AN INTERSPECIFIC BREEDING PROGRAMME IN COTTON ASSISTED BY RAPD MARKERS

Mergeai, G., Bi Irié V., Dujardin P. and Baudoin J.P.

Faculté des Sciences Agronomiques de Gembloux, Belgium

INTRODUCTION

The transfer into cultivated cotton of interesting traits from distant wild species is almost automatically accompanied by undesirable characters (Hau, 1981). The amount of foreign genetic material introgressed into the cultivated parent depends on the affinities existing between the two genomes implicated in the breeding process (Louant et al., 1977). Several generations of backcrossing are generally necessary to restore a normal fertility of the introgressed material. Molecular markers such as RAPD can be very useful to accelerate this process of purification by helping to choose among the progeny of each backcross the genotypes that are genetically the closest to the cultivated parent (Paterson et al., 1991).

This strategy is followed in Gembloux in the frame of an interspecific breeding programme involving *G. hirsutum* and Australian wild diploid species. The objective of this programme is the introgression into *G. hirsutum* of the glanded-plant and glandless seed trait of Australian cottons.

Prerequisites for the use of RAPD markers are an efficient extraction procedure for cotton DNA and a procedure for RAPD reactions to be carried out with this DNA. The last two years were devoted to optimise these protocols.

Development of a new DNA extraction protocol .

After modifying different parameters of the classic CTAB DNA extraction method (Murray & Thompson,1980), we succeeded to develop a new protocol (Irie et. al., 1995) which gives very good results and can be used with rather old cotton leaves. This method is also efficient for other recalcitrant species including date palm, rubber tree, cassava, banana and coffee. The main improvement brought to the CTAB method concerns the addition of active charcoal to eliminate secondary metabolic products. When active charcoal is added just before the incubation at 60°C, we generally obtain 75 to 150 µg DNA/g fresh weight leaf, with A_{260}/A_{280} = 1,7 to 2,0. This clean DNA is easily dissolved in T.E. and is completely digested by RFLP enzymes (EcoRI, EcoRV, BamhI, ScaI, SacI, etc.). The extracted DNA is totally usable for RAPD reactions.

Optimisation of the RAPD reactions with cotton DNA

RAPD reactions have been optimised with the cotton DNA produced by the new extraction method. Using a TECHNE.PHC3 thermocycler, we brought several improvements to the protocol advised by Operon technologies (Williams et. al., 1990) and to the PCR amplification protocol of a rbcs (rubilose bicarboxylase small unit) gene. The main parameters we improved concern : the duration of the first denaturation, the annealing temperature, the number of amplification cycles and the concentration of reagents ($MgCl_2$, dNTPs, primers and DNA).

All the tests have been realised with the following primers: OPE-01, OPE-02, OPE-03, OPE-04, OPE-05 and OPE-06. The optimal RAPD conditions for cotton DNA are: first denaturation : 94°C during 5 min; amplification: 45 cycles of 1 min at 94°C; annealing : 1 min at 34°C, elongation: 2 min at 72°C; final elongation : 7 min 30 sec at 72°C until the end of the reaction. The best amplification are carried out in 25 µL reactions with 50 ng template DNA, 1 U Taq Polymerase, 2 mM MgCl2, 200 µM of each dNTP (A, G, C, T) and 20 picoM of random primer. These improved conditions are also more or less suitable for RAPD reaction with DNA extracted from coffee, rubber tree, cassava and banana.

Production of tetraploid cotton plants showing the glandless-seed and glanded plant trait

Two trispecific synthetic allotetraploids have been obtained using the Australian diploid species *G. sturtianum*, the main cultivated amphidiploid, *G. hirsutum*, and two American wild diploid species : *G. thurberi* and *G. raimondii*. Observation in the progeny of these trispecific hybrids revealed the expression of the gossypol glands morphogenesis repressive mechanism of *G. sturtianum* in a rather high proportion of the BC1 seeds (6 on 41). In these materials, the glandless-seed and glanded-plant trait seems to be linked to a lethal factor. Only one of the six totally glandless BC1 seeds gave an adult plant. In vitro culture of the seed and grafting of the plantlet at a early stage on *G. hirsutum* were necessary to obtain a normal development of this genotype. All the other glandless materials died just after germination or never germinated. The survival plant will be used in a backcrossing programme to obtain the introgression of the glanded-plant and glandless-seed character into *G. hirsutum* (Mergeai et. al., 1995).

CONCLUSION AND PERSPECTIVES

During the last two years the main activities of our laboratory have concerned the development of a new DNA extraction method applicable to cotton, the optimisation of the RAPD reactions realisable with this DNA and the identification of RAPD polymorphic markers usable to accelerate the selection of commercial plants in the progeny of two trispecific hybrids (*Thurberi - Sturtianum - Hirsutum* and *Hirsutum - Raimondii-Sturtianum*). Five useful markers have been identified.

In the same time we continued an interspecific hybridization programme aiming at the introgression of the glandless-seed and glanded-plant trait from *G. sturtianum* into *G. hirsutum*. In the progeny of a *Thurberi-Sturtianum-Hirsutum* trispecific hybrid we obtained a totally glandless tetraploid BC1 seed that gave rise to a glanded plant. This material will be backcrossed by *G. hirsutum* and its progeny will be analysed with the help of the RAPD polymorphic markers that are currently developed in our laboratory.

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