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SHORT COMMUNICATION

Use of RAPD-PCR to identify true hybrid plants from crosses between closely related progenitors

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ABSTRACT

RAPD-PCR molecular markers were used to identify common bean and soybean hybrid plants derived from crosses between closely related progenitors, with no apparent phenotypic differences. Primers OP-F12 and OP-C03 were used to identify true hybrids derived from crosses between common bean cultivars Rudá (A 285) and AN 910408, and soybean cultivars Cristalina and Bossier, respectively. Each primer generated one polymorphic DNA band which was present in the male progenitor and absent in the female progenitor. As RAPD bands are normally inherited as dominant characters, the presence of these bands in the F_1 plants confirmed their status.

INTRODUCTION

Molecular markers can be extremely useful in plant breeding to solve practical problems faced by the breeder. One typical example of such use is the confirmation of crosses between closely related cultivars which cannot be easily distinguished phenotypically.

In our backcross breeding program for the creation of common bean cultivars resistant to anthracnose one of the donor parents is AN 910408, a "carioca"-type cultivar, resistant to races 73, 89, 67, 83, 343, and 339 of *Colletotrichum lindemuthianum*, but with a low yield potential. The recurrent parent, cultivar Rudá (A 285), is also "carioca" type, with a good yield potential but susceptible to most races of anthracnose. Unfortunately these cultivars cannot be distinguished phenotypically, making the identification of true hybrid plants difficult, particularly during the summer season when the number of successful crosses decreases considerably. The same situation is true for the cross between soybean progenitors Bossier and Cristalina, which are used in our backcross breeding program for resistance to cercosporiosis (*Cercospora sojina* Hara) in soybean.

The aim of this work was to test the feasibility of using PCR-based molecular markers (Williams *et al.*, 1990) to identify true hybrid plants derived from phenotypically similar progenitors.

MATERIAL AND METHODS

Common bean cultivars AN 910408 and Rudá, and soybean cultivars Bossier and Cristalina were crossed in the greenhouse under controlled

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environmental conditions. In both cases the first cultivar was used as pollen donor. These crosses are part of the backcross breeding programs for resistance to anthracnose in common bean and to cercosporiosis in soybean that are presently being conducted at the Universidade Federal de Viçosa, Viçosa, MG, Brazil.

Leaf DNA was extracted from the common bean and soybean progenitors, and from the potential F_1 plants by a mini-prep procedure based on Doyle and Doyle (1990). Amplification reactions of 25 µl contained 25 ng of DNA, 0.1 mM of each dNTP, 2.0 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.4 µM of primer OP-F12 or OP-C03 (Operon Technologies, Alameda, CA, USA), and one unit of Taq DNA polymerase. Reactions were performed in a thermocycler model 9600 (Perkin-Elmer) programmed for 40 cycles, each consisting of one denaturation step (94°C for 15 sec), one annealing step (35°C for 30 sec), and one extension step (72°C for 60 sec). The final cycle was followed by a extension step at 72°C for 7 min. The amplification products were analyzed on 1.2% agarose gels immersed in TBE buffer (90 mM Tris-borate, 1 mM EDTA, pH 8.0). DNA bands were stained with ethidium bromide (10 µg/ml), and visualized under UV light.

RESULTS AND DISCUSSION

Preliminary results from our laboratory suggested that polymorphic DNA bands could be detected between common bean cultivars AN 910408 and Rudá, and between soybean cultivars Bossier and Cristalina. Indeed, our results demonstrated that primer OP-F12 generated a RAPD band of approximately 940 base pairs which was present in the male common bean progenitor (AN 910408) and absent in the female progenitor (Rudá). In addition, primer OP-C03 revealed a band (~780 bp) which was amplified in the soybean male progenitor Bossier but not in the female progenitor Cristalina. As RAPD bands are usually inherited in a dominant fashion, DNA samples from potential F_1 plants derived from these two crosses were amplified with the appropriate primers. The ones harboring the band present in the male progenitor were considered true hybrids (Figures 1 and 2). About 30% of the plants analyzed did not present the expected bands and were discarded since they were probably derived from self-pollination.

The methodology tested proved to be fast and reliable, saving time and greenhouse space, and most of all ensuring that only true hybrid plants are used for backcrossing.

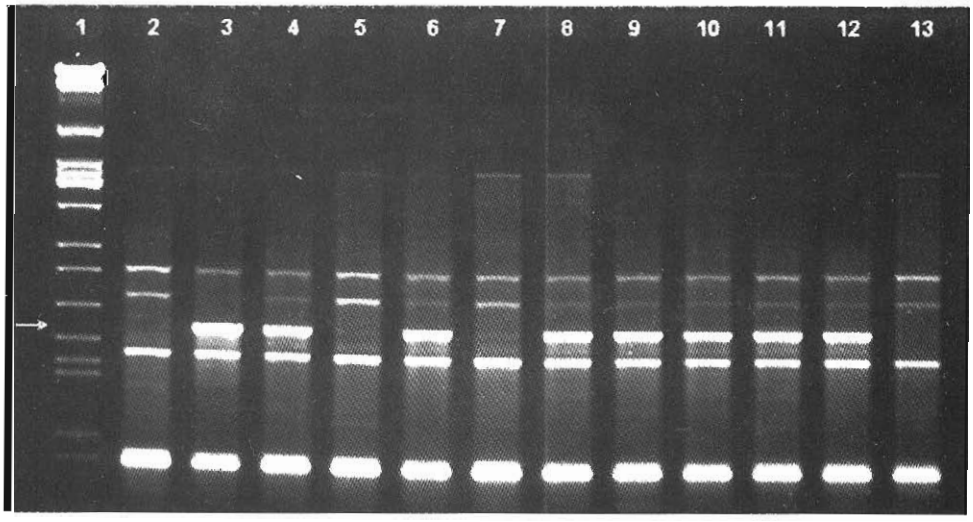

Figure 1 - Electrophoretic analysis of DNA amplification products from common bean cultivars Rudá, AN 910408, and potential hybrids derived from crosses between them. Amplifications were performed with primer OP-F12. Lanes are as follows: 1, lambda bacteriophage DNA digested with *Eco*RI, *Bam*HI and *Hin*dIII (size markers); 2, cv. Rudá; 3, cv. AN 910408; 4-13, potential hybrids. Arrow indicates a 940-bp band which is polymorphic between the progenitors.

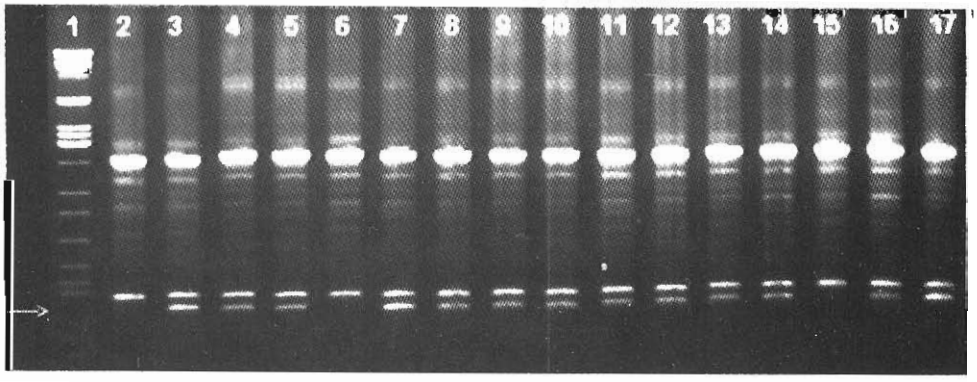

Figure 2 - Electrophoretic analysis of DNA amplification products from soybean cultivars Cristalina, Bossier, and potential hybrids derived from crosses between them. Amplifications were performed with primer OP-C03. Lanes are as follows: 1, lambda bacteriophage DNA digested with *Eco*RI, *Bam*HI and *Hin*dIII (size markers); 2, cv. Cristalina; 3, cv. Bossier; 4-17, potential hybrids. Arrow indicates a 780-bp band which is polymorphic between the progenitors.

ACKNOWLEDGMENTS

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RESUMO

Marcadores do tipo RAPD-PCR foram usados na identificação de híbridos, tanto de soja como de feijoeiro, derivados de cruzamentos entre progenitores que não contrastavam para características facilmente monitoráveis. Os oligonucleotídeos iniciadores OP-F12 e OP-C03 foram usados com o objetivo de identificar híbridos verdadeiros derivados de cruzamentos entre as variedades de feijoeiro comum Rudá (A 285) e AN 910408, e de soja Cristalina e Bossier, respectivamente. Cada um desses iniciadores gerou uma banda polimórfica de DNA, presente no progenitor masculino e ausente no progenitor feminino.

REFERENCES

Doyle, J.J. and **Doyle, J.L.** (1990). Isolation of plant DNA from fresh tissue. BRL *Focus 12*: 13-15.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and **Tingey, S.V.** (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res. 18*: 6531-6535.

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