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ESTABLISHMENT OF A MICROMETHOD FOR GENOME CHARACTERIZATION OF *OPUNTIA FICUS-INDICA* BY RAPDs

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Summary

A micromethod for DNA extraction and RAPD analysis was established for genome characterization of *Opuntia ficus-indica* plants. 100 mg of the chlorenchyma of cladodes are appropriate to give high yields of DNA. Up to 100 µg DNA of high molecular weight per g fresh weight were achieved. RAPD analyses was performed with 10 ng template DNA. The method gives highly repeatable results for RAPD analysis and is appropriate to evaluate homogeneous as well as polymorphic plant material.

Introduction

Availability of a rapid and efficient method for genome characterization of *Opuntia ficus-indica* is extremely important for the cactus community to perform studies on genetic diversity and to develop efficient breeding methods. Difficulties to establish a micromethod for this species are mainly due to the extraordinarily high content of mucilage in all plant organs. Existing methods use 2 to 8 grams of fresh material (De la Cruz et al., 1997, Wang et al., 1998, Mondragon-Jacobo et al., 2000). Additionally, reproducibility is a well-known problem of genome characterization by the RAPD technique. Besides unsatisfactory technical standardization, the use of different tissues or organs consisting of various amounts of different tissues as well as the inclusion of plants of different ages into experiments may also be a reason for problems with the repeatability of RAPD fingerprints (Chen et al., 1997, Schaefer et al., 2000, Schaffer and Arnholdt-Schmitt, 2001).

Results

A modified protocol of the kit "Nucleon Phytopure for Plant DNA Extraction" (Amersham Pharmacia Biotech) is applied on 100 mg of explants of the chlorenchyma tissue of cladodes of *O. ficus-indica* plants. Various extractions of plants from the same accession gave comparable results of high molecular weight DNA (as an example see Fig.1). By comparing the DNA of the defined tissue, i.e. the chlorenchyma, it was possible to succeed in highly repeatable results by RAPD analysis. For RAPD investigation, a kit (Ready-to-go RAPD analysis beads, Amersham Pharmacia Biotech) was applied, which contains two polymerases and reduces the number of steps for pipetting. Experiments to analyse various plant genotypes for homogeneity or polymorphism were performed (see Arnholdt-Schmitt et al., in press). In Fig. 2 an example is presented to demonstrate polymorphism between a fruit producing and a fodder crop genotype.

Conclusion

The presented micromethod will now be available for tests in screening studies on *Opuntia ficus-indica* genotypes. Advantages and disadvantages of the method are discussed in more detail in Arnholdt-Schmitt et al. (in press).

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