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The assessment of genetic stability of *Magnolia x soulangiana* and *Magnolia stellata* plant regenerated *in vitro* using RAPD markers

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Abstract The objective of this study was to determine the genetic stability of *Magnolia stellata* and *Magnolia x soulangiana* microshoots regenerated *in vitro*. For this purpose five primers were used (from 12 previously tested) and the similarity between RAPD profiles corresponding control plant respectively microshoots regenerated *in vitro*, was interpreted as suggesting genetic stability.

Key words

Magnolia x soulangiana, Magnolia stellata, in vitro, genetic stability, RAPD

Magnolias are species of trees and shrubs with great ornamental value by large flowers, brightly colored, which usually occurs before leaf emergence.

In situ multiplication is performed sexually by seed and asexually by vegetative tissues (layering, grafting and cuttings). Magnolias multiplication by conventional methods raises a number of problems, both in terms of obtaining and the characteristics of saplings obtained. An advantageous alternative that enables the rapid provision of high quality planting material is *in vitro* propagation who has a number of advantages both technical and economic. Magnolias micropropagation was achieved from shoot tips and axillary buds [1, 4] and via somatic embryogenesis [5].

Propagation through *in vitro* regeneration of plants has some disavantages [7], the most important being the associated genetic instability.

Brown and others (1993) proposed the use of PCR for assessment of genetic fidelity of plants obtained by tissue culture, RAPD technique being successfully used for genetic analysis of plant material regenerated *in vitro* [6].

Tehnique RAPD was first described by Williams and others (1990) and is based on the allelic polymorphism at the parental forms in the presence or absence of amplifications products. Generally each primer used will determine the amplification of sequences from different loci of the genome, provides an effective method to investigate DNA polymorphisms between individuals. Tehnique RAPD applications are: study of genetic diversity, germplasm characterization, determining the genetic structure of populations, somaclonal variability, identification of cultivars and hybrids purity [3].

Material and Method

Biological material

Donor plants from which we are taken explants with which were initiated *in vitro* cultures with minishoots obtained after several successive subcultures on the multiplication medium were used as biological material for studying genetic stability in *in vitro* culture of *Magnolia x soulangiana* and *Magnolia stellata* species. For each species, we studied 5 lines regenerated from 5 different apical bud explants. DNA was extracted from 10 regenerated of each selected line. From the isolated DNA samples under each line we made a mixture sample who was compared to mother plants. In total, 100 plants were investigated.

Working method

To confirm the genetic stability of *in vitro* regenerated plants was used RAPD technique.

For genomic DNA extraction was used automatic nucleic acids extraction apparatus Maxwell ™ 16 Instrument from Promega.

Mixture for obtaining amplification products consisted of: GoTaq ® Green Master Mix Kit 2x (Promega) composed of GoTaq ® DNA polymerase, dNTP, $MgCl_2$, reaction buffer, 10 pmol/ml primer (Fermentas), 50-100 ng genomic DNA, distilled water, free of nucleases (table 1).

Table 1

The composition of amplification mixture for RAPD markers

Components	Quantity (1x)
Green Master Mix	12,5 µl
Sterile distilled water	9 µl
Primer	1,5 µl
DNA	2 µl

In these experiments we used 12 RAPD primers (table 2).

Table 2

RAPD primers used to assess the variability of genotypes considered for the study

Code	Sequence (5'- 3')	Code	Sequence (5'-3')
OPA-13	CAGCACCCAC	OPC-19	GTTGCCAGCC
OPA-11	CAATCGCCGT	OPA-09	GGGTAACGCC
OPB-03	CATCCCCCTG	OPB-01	GTTTCGCTCC
OPA-17	GACCGCTTGT	OPW-04	CAGAAGCGGA
OPC-18	TGAGTGGGTG	OPW-09	GTGACCGAGT
OPC-14	TGCGTGCTTG	OPB-02	TGATCCCTGG

Amplification was performed in Corbett thermocycler. Conditions for PCR amplification were: 4 minutes at 94^0C initial denaturing, followed by a total of 45 cycles, each with the following steps: 3 min denaturing at 94^0C, 1 min at 36^0C attaching primers, 2 min extension and 3 minutes, final extension at 72^0C. The amplification products were separated in agarose gel concentration of 2% in TAE 1X buffer at a voltage between 80 V and 100 V for a duration of 40-60 minutes and were analyzed by coloring with ethidium bromide.

DNA from the gel was visualized using a UV transilluminator and photographed with the camera PHOTODOCUMENTATION SYSTEM Model: DP-001. FDC.

Obtained results

Only five of the 12 RAPD primers tested generated measurable bands to regenerated of genotypes studied: OPA-11, OPB-03, OPC-19, OPW-04, OPW-09.

In the case of *Magnolia x soulangiana* species, RAPD primers selected generated a total of 19 bands that were monomorphic for all plants analyzed, including the control plant. The number of bands for each primer varied between 3 (OPC-19) and 4 (OPA-11, OPB-03, OPW-04, OPW-09), with an average of 3,8 bands per primer. Regarding the size of amplified DNA fragments, it varied between 1100 bp and 250 bp (table 3).

Table 3

List of primers, their sequence and size of the amplified fragments generated by 5 RAPD primers

Crt. no.	Primer	Sequence (5'- 3')	Scorable bands (no.)	Monomorphic bands (no.)	Size (bp)
1	OPA-11	CAATCGCCGT	4	4	1100, 700, 600, 500
2	OPB-03	CATCCCCCTG	4	4	750, 500, 400, 350
3	OPC-19	GTTGCCAGCC	3	3	700, 630, 520
4	OPW-04	CAGAAGCGGA	4	4	830, 720, 400, 320
5	OPW-09	GTGACCGAGT	4	4	750, 340, 300, 250

RAPD analysis of plants regenerated *in vitro* from the *Magnolia x soulangiana* species showed a profile identical to mother plants, which indicates that genetic changes were not induced. In all cases RAPD profiles

of regenerated plants were monomorphic and similar to mother plants.

Examples of RAPD patterns amplified with primers OPA-11, OPB-03, OPC-19, OPW-04 and OPW-09 are shown in figures 1-3.

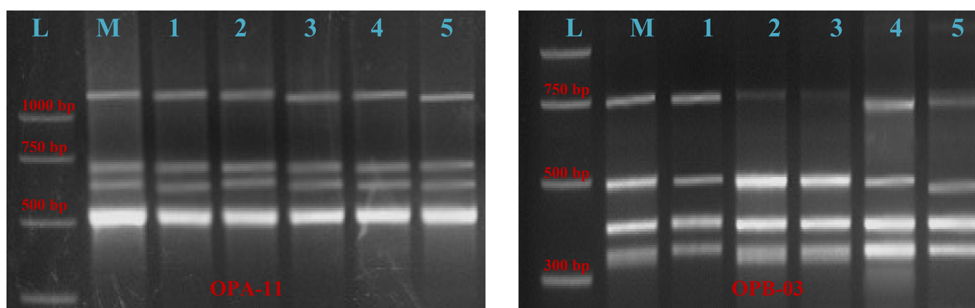

Fig. 1. RAPD profiles generated by primer OPA-11 (left) and OPB-03 (right): L = DNA marker, M = mother plant; 1-5 = samples mixture of regenerated plants under each selected line

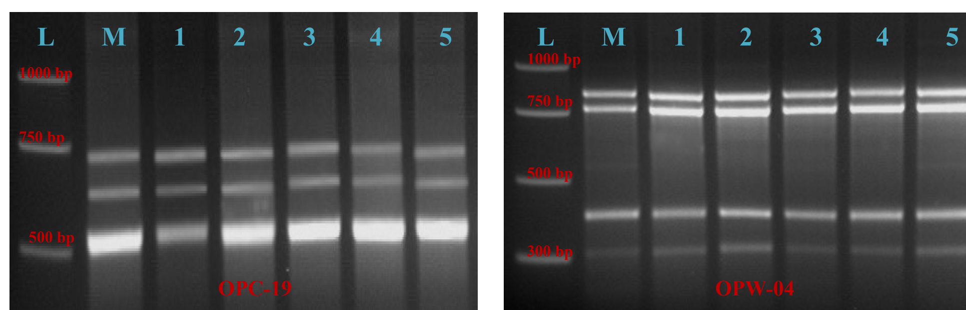

Fig. 2. RAPD profiles generated by primers OPC-19 (left) and OPW-04 (right): L = DNA marker, M = mother plant; 1-5 = samples mixture of regenerated plants under each selected line

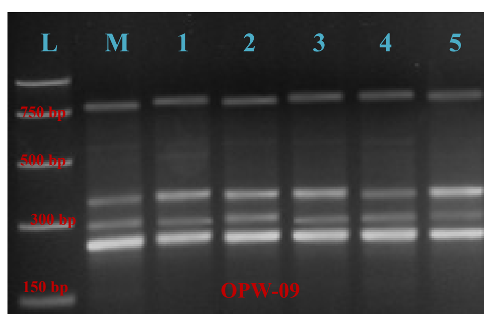

Fig. 3. RAPD profiles generated by primer OPW-09: M = DNA marker, M = mother plant; 1-5 = samples mixture of regenerated plants under each selected line

Because no were identified changes in the number and distribution in DNA gel bands obtained after amplification of RAPD markers tested when compared the mother plant with regenerated plants, we conclude that the use of *in vitro* culture for *Magnolia x soulangiana* plant regeneration can be applied without risk of induction and expression of genetic instability.

Similarly, *Magnolia stellata* plants regenerated *in vitro* were studied in terms of genetic stability.

At the *Magnolia stellata* species, selected primers generated a total of 21 bands also monomorphic, and the number of bands per primer varied between 4 (OPA-11, OPB-03, OPC-19, OPW-09) and 5 (OPW -04), with an average of 4,2 bands per

primer. Amplified DNA fragment size varied between 1000 bp and 200 bp (table 4).

Table 4

List of primers, their sequence and size of the amplified fragments generated by 5 RAPD primers

Crt. no.	Primer	Sequence (5'- 3')	Scorable bands (no.)	Monomorphic bands (no.)	Size (bp)
1	OPA-11	CAATCGCCGT	4	4	730, 520, 450, 300
2	OPB-03	CATCCCCCTG	4	4	1000, 970, 700, 530
3	OPC-19	GTTGCCAGCC	4	4	600, 400, 300, 200
4	OPW-04	CAGAAGCGGA	5	5	650, 500, 400, 350, 230
5	OPW-09	GTGACCGAGT	4	4	500, 400, 350, 230

RAPD profiles revealed by primers OPA-11, OPB-03, OPC-19, OPW-04 and OPW-09 were monomorphic in all plants from *in vitro* culture, being similar to the mother plant pattern (fig. 4-6).

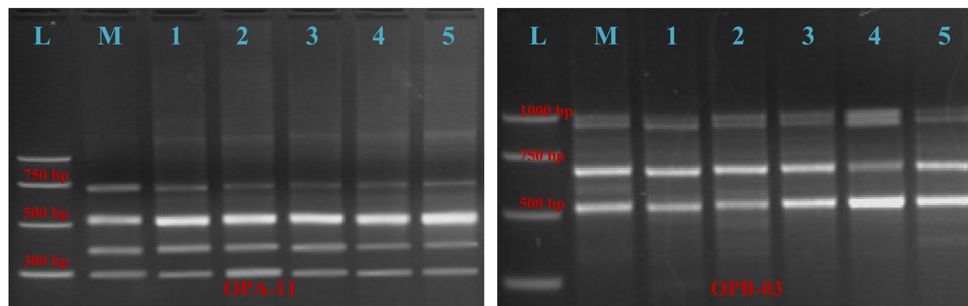

Fig. 4. RAPD profiles generated by primer OPA-11 (left) and OPB-03 (right): L = DNA marker, M = mother plant; 1-5 = samples mixture of regenerated plants under each selected line

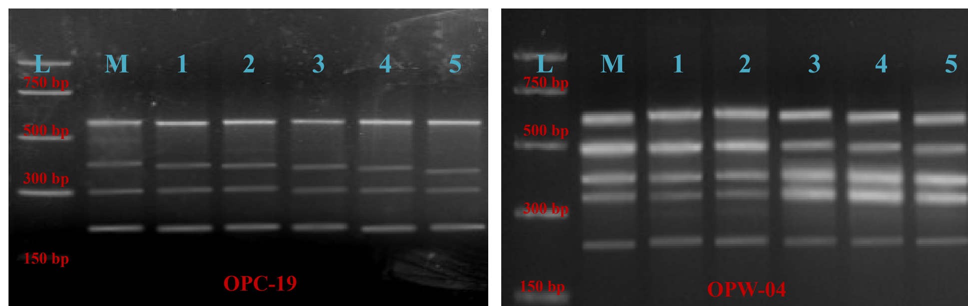

Fig. 5. RAPD profiles generated by primers OPC-19 (left) and OPW-04 (right): L = DNA marker, M = mother plant; 1-5 = samples mixture of regenerated plants under each selected line

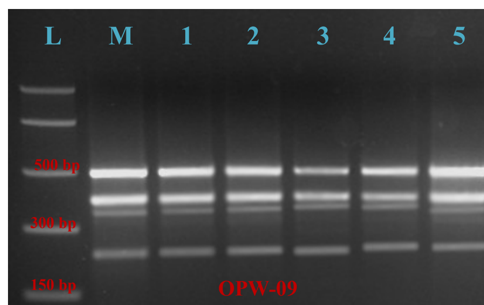

Fig. 6. RAPD profiles generated by primer OPW-09: M = DNA marker, M = mother plant; 1-5 = samples mixture of regenerated plants under each selected line

These results led to the conclusion that even in the case of *Magnolia stellata* species, *in vitro* cultured can be used as a method of multiplication without risks of changes at the molecular level.

Conclusions

1. Lack of polymorphic bands between RAPD profiles of microshoots regenerated *in vitro* and mother plants, shows that there has been any genetic variation.

2. Because between RAPD patterns of genomic DNA isolated from plants regenerated *in vitro* there were no changes compared to mother plants, we conclude that for the *Magnolia stellata* and *Magnolia x soulangiana* species *in vitro* culture can be used as a method of multiplication without risk of occurrence of changes at the molecular level.

3. The similarity between RAPD profiles may be associated with high genetic stability of these genotypes.

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