УДК 57.085.2:001.8:582.671

ISSR-ANALYSIS OF GENETIC HOMOGENEITY OF PLANTS-REGENERANTS OF MAGNOLIA KOBUS DC.

O. Dubin, candidate of agricultural sciences
I. Boboshko-Bardin, candidate of agricultural sciences

Genetic uniformity of regenerated plants Magnolia kobus DC was studied. According to five ISSR-primers $(ACC)_6G$, $(CTC)_6A$ $(AGC)_6G$, $(TCG)_6G$, $(GTG)_6A$ and $(GAG)_6G$ have been researched. The identity between plants-donors, explants and adapted sapling-regenerants have been confirmed. High genetic stability of genomes Magnolia Kobus by its microclonal reproduction its in vitro has been proved.

Microclonal reproduction, plant-donor, plant-regenerant, somaclonal variation, PCR, ISSR - PCR marker, genome.

Tissue culture is a unique experimentally established biological system, representing the population of dedifferentiated somatic cells capable of regenerating various organs and even the whole system [2, 4, 5]. It is widely used in research on genome organization and functioning in the process of regeneration, adaptation to stressful growth conditions, while maintaining, improvement and rapid reproduction of valuable genotypes. At the chromosomal level somaclonal variability has been researched for a long time.

The high genetic variability of woody plants causes significant polymorphism of plants of one type by their monumentality, energy, growth and other characteristics. Quantitative and qualitative mutations, cytological abnormalities, DNA sequence changes, activation and termination of genomes expression occur in cells, tissues and organs of plants in vitro culture, and regenerated plants [6]. Such variability has a practical value for selection since it is selection a source of genetic diversity, which is used to provide the plants with

desirable traits (increase of decoration, changes of flowering time, resistance to diseases and pests, herbicides, salt-, frost - and drought-resistance, resistance to other abiotic stresses [9].

Now to solve a number of theoretical and practical issues special attention is paid to molecular-genetic methods of research. In selection of ornamental woody plants research using molecular genetic markers is being actively implemented [1, 3, 6, 8]. Using the latest research methods and DNA technologies open new opportunities in genetics and selection of ornamental plants and will allow to significantly extend the study of the genetic structure of populations of ornamental woody plants.

Molecular genetic methods based on polymerase chain reaction (PCR) are widely used. One of the directions of PCR which not require knowledge of the primary structure of DNA is ISSR (*Inter Simple Sequence Repeat PCR*). This type of markers was not only a convenient and informative, but also a reliable tool to identify genetic variability of individuals and various taxonomic units.

The aim of our study was to test the genetic homogeneity of plants donor Magnolia kobus and individuals in aseptic culture in vitro by using ISSR markers.

Material and methods of research. Material used for research were: leaves of donor trees *Magnolia kobus* DC. from the National botanic garden of Grishko national Academy of Sciences of Ukraine; aseptic culture obtained by microclonal reproduction and plants regenerants obtained by direct morphogenesis and adapted to the conditions *in vivo*.

Genomic DNA was extracted from the leaf disks of plants by STAB-method [7]. Polymerase chain reaction was carried out on amplifer Tertsik (DNA-technology, Russia [7]) according the following temperature conditions: initial denaturation continued 4 minutes at 94 °C; 32 cycles: 30 sec at 94 °C, 30 sec at 58 °C, 2 minutes at 72 °C; terminal elongation - 5 minutes at 72 °C.

The reaction mixture with a volume of 20 ml contained: 67 mM Tris-HCl (pH 8,8), 17 mM $(NH4)_2SO_4$, 0,01 % Tween-20, 0,2 mM dNTP, 1 unit of Tag- polymerase, 40 ng of genomic DNA, 2,0 mM $MgCl_2$ and 0,4 mkM primer. 12

ISSR-primers with trinucleotide core sequences and one anchor nucleotide on the 3' -end (tab. 1) were used.

Electrophoretic separation of amplification products was performed in 2 % agarose gel using 1×TBE buffer. After electrophoresis gel was treated with methyl ethidium (5 mg/ml) and PCR-products were photographed by digital camera. The molecular weight of PCR-products was determined by the marker GeneRuller 100 bp (Fermentas).

1. Nucleotide sequence of the used ISSR-primers

№	Primer	Nucleotide sequence, 5→3
1	(ACC)6G	ACC ACC ACC ACC ACC ACC G
2	(CTC)6A	CTC CTC CTC CTC CTC CTC A
3	(TCG)6G	TCG TCG TCG TCG TCG TCG G
4	(AGC)6G	AGC AGC AGC AGC AGC AGC G
5	(TCG)6G	TCG TCG TCG TCG TCG TCG G
6	(GTG)6A	GTG GTG GTG GTG GTG GTG A
7	(CTC)6C	CTC CTC CTC CTC CTC CTC C
8	(GAG)6G	GAG GAG GAG GAG GAG GAG G
9	(ACC)6G	ACC ACC ACC ACC ACC ACC G
10	(AGC)6C	AGC AGC AGC AGC AGC AGC C
11	(GCT)6A	GCT GCT GCT GCT GCT GCT A
12	(CCA)6G	CCA CCA CCA CCA CCA CCA G

The results of the research. In order to identify informative molecular and genetic markers which would allow to analyze the magnolia genome changes in microclonal reproduction conditions in aseptic culture, at the first stage of study a screening of 12 ISSR-primers with trinucleotide core sequences was carried out (fig. 1). According to the picture of amplification of the used primers the obtained initial specters had the following characteristics: lack of amplification products (CCA)6G); diffuse specters without clear discrete bands ((TCG)6G, (CTC)6C and (AGC)6C); specters with insufficient number of amplicons for further analysis ((AGC)6G and (GCT)6A) and specters with clear PCR-products (ACC)6G, (CTC)6A (AGC)6G, (TCG)6G, (GTG) (6A) and (GAG)6G).

Optimization of conditions of polymerase chain reaction in the first three cases (changes of the concentration of regents of the reaction mixture and the variation of temperature of annealing primers) did not lead to a significant improvement of the specters. Therefore testing of genetic homogeneity of

magnolia plants was performed using primers with nucleotide sequences: (ACC)6G, (CTC)6A (AGC)6G, (TCG)6G, (GTG) (6A) and (GAG)6G.

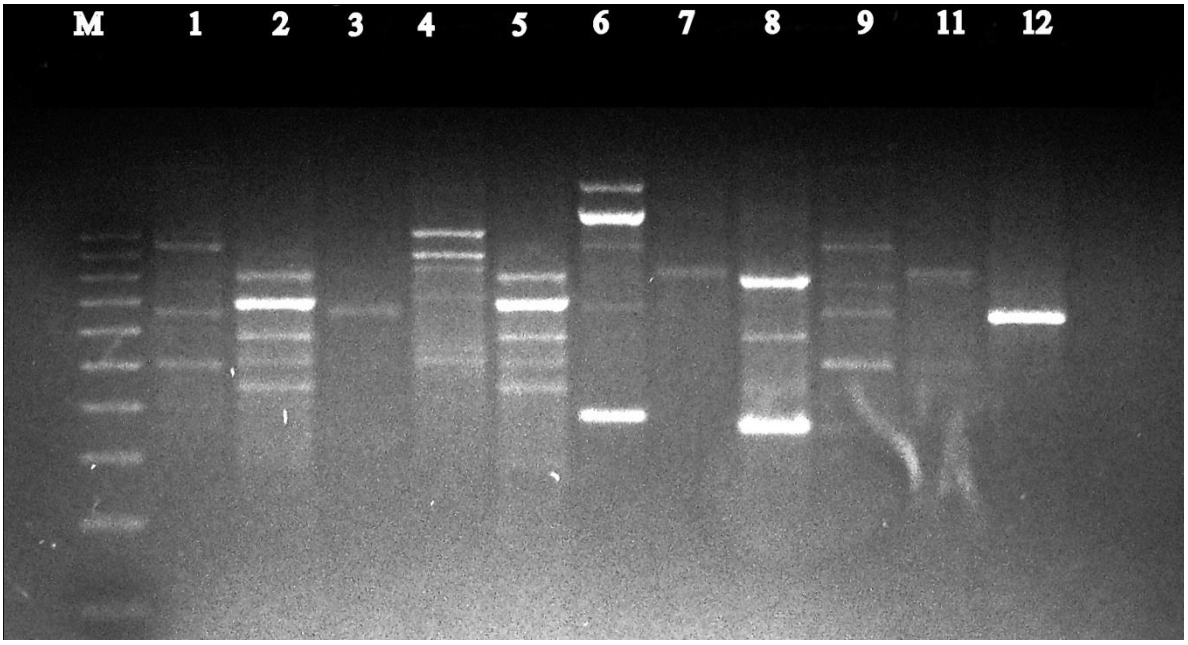

Fig. 1. Specters of amplification products ISSR-PCR at primary screening of primers: M – marker of molecular weight; 1-12 - tested primers.

Molecular-genetic analysis of tissue cultures, organs and adapted sapling-regenerants M. kobus using of the method ISSR-PCR with primers (ACC)6G, (CTC)6A (AGC)6G, (TCG)6G, (GTG) (6A) and (GAG)6G showed absolute identity between the relevant plants donors, explants and adapted sapling - regenerants (fig.2).

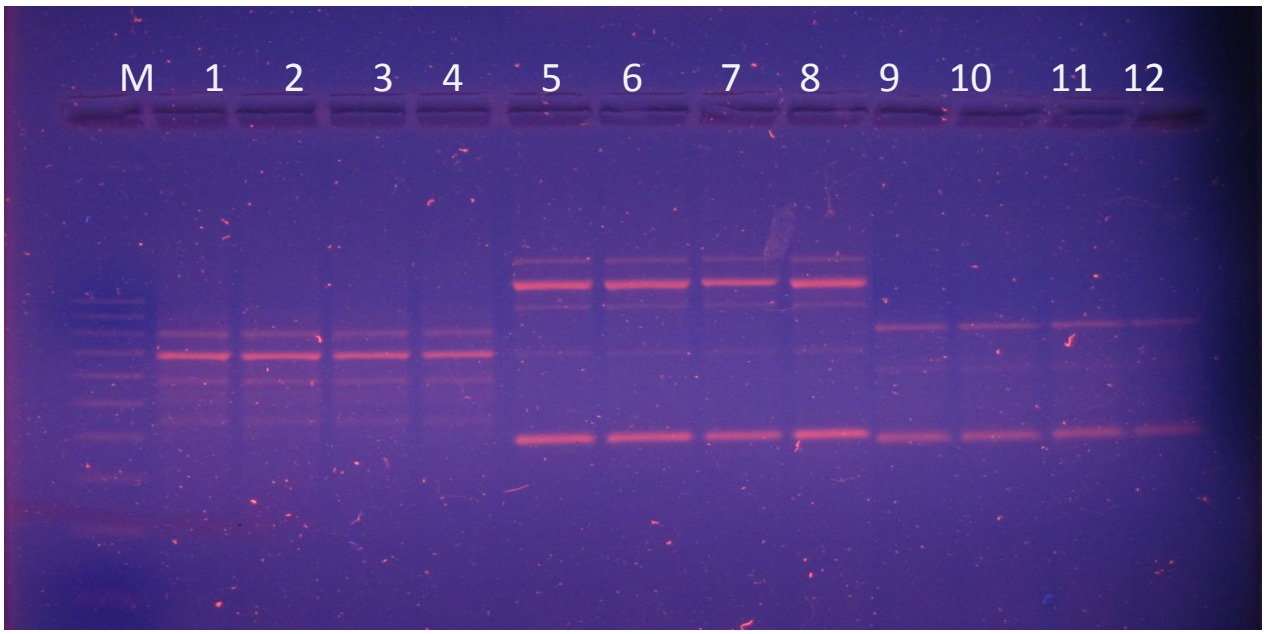

Fig. 2. Electrophoretic separation of amplification products of ISSR-PCR with primers (TCG)6G, (GTG) (6A) and (GAG)6G: 1, 5, 9 – donor plant; 2, 6, 10 – plant-regenerant; 3, 4, 7, 8, 11, 12 – adapted sapling-regenerant; M – molecular weight marker (GeneRuler 1kb DNA Ladder, GeneRuler 100 bp DNA Ladder (Fermentas)).

Conclusion. The results obtained testify the relative genetic stability of magnolia kobus genomes by microclonal reproduction in vitro and give opportunies to use the obtained cultures of tissues and organs with the purpose of preservation of the gene pool of this species and decorative forms, production of large quantities of plant material with the desired characteristics and properties.