بررسی پایداری ژنتیکی گیاهان ریز افزایی شده *Araucaria excelsa* R. Br. var. *glauca* با استفاده از انگشت نگاری نشانگر مولکولی RAPD

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هدف کار حاضر نه تنها بررسی ریز افزایی این گیاه با تاکید بر تنظیم کننده های رشد 2iP، BA، TDZ و کینتین بود، بلکه پایداری ژنتیکی گیاهان کشت بافتی به دست امده از گیاه مادری با استفاده از انگشت نگاری نشانگر مولکولی RAPD مورد ارزیابی قرار گرفت. بدون در نظر گرفتن تاثیر موقعیت ریزنمونه، ۰/۵ میکرومولار 2iP منجر به میانگین پرآوری ٤ و طول شاخساره ۷/٦٥ میلی متر شد. استفاده از ۰/۰٤٥میکرومولار TDZ منجر به میانگین پرآوری ٤/٦۰ و میانگین طول شاخساره ۷/۰۸ میلی متر شد. ریزنمونه های رشد یافته در غلظت های بیش از ۰/۵ میکرومولار BA و 2iP و همچنین ریزنمونه های گرفته شده از قسمت های پایینی ساقه عمودگرای کاج مطبق باوجود پرآوری خوبی که در بعضی موارد نشان دادند در پایان رشدشان متوقف شده و از بین رفتند. ارزیابی نشانگر مولکولی RAPD بیانگر این است که ۲۵۳۱ نوار از ۱۲ پرایمر RAPD به‌دست آمد. تعداد مکان های ژنی از ۹ تا ۲۳ متغیر بود. واکاوی داده های RAPD با استفاده از UPGMA ۹۲٪ شباهت را بین گیاهان ریز افزایی شده و گیاه مادریشان با استفاده از ضریب تشابه جاکارد نشان می دهد. ماتریکس تشابه و PCOA نیز نتایج یکسانی داشت که بیانگر شباهت بسیار زیاد گیاهان رشد یافته درون شیشه ای و گیاه مادری است. پرایمرها تنها ۳۲٪ چند شکلی را نشان دادند. زیرکشت شاخساره های پرآوری کرده در محیط کشت MS دارای ۷/۵ میکرومولار IBA و NAA برای ۱۵ روز پیش از انتقال به محیط نیم غلظت MS بدون تنظیم کننده رشد منجر به ریشه زایی ۳۳٪ با یک تا دو ریشه در هر ریزنمونه شد. گیاهک ها سرانجام در آمیخته پرلایت و پیت در شرایط رطوبت ۹۵٪ نگهداری و سپس به گلخانه منتقل شدند.

کلمات کلیدی: دی. ان. ای چند شکل تکثیر شده تصادفی، پایداری ژنتیکی، کاج مطبق، ریز افزایی

Introduction

The most important part of any *in vitro* propagation system is mass multiplication of plantlets that are genetically homogenous and phenotypically uniform. Generally, in callus culture and through adventitious shoot formation some desirable characters may be lost because of somaclonal variation occurrence. Several approaches have been applied for identifying variants among micropropagated plants. Molecular markers have been shown to enhance breeding efforts in annual and perennial crops, since they are not altered by major environmental factors. The aim of the present work was the possible finding of genetic variability in micropropagated plants derived from orthotropic stem explant in *Araucaria excelsa* R. Br.

Materials and methods

Three year's old seedlings of *A. excelsa* R. Br. var. glauca were chosen for the present study. Total DNA was extracted from 100 mg leaves of 13 lines of micropropagated plants and their donor mother plants. PCR was performed in a volume of 20 µl containing 8 ng of template DNA, 10 µM of decamer primers, 1 × reaction buffer, 2.5 mM $MgCl_2$, 0.1 mM dNTP mix and 2 U Taq DNA polymerase (The optimized PCR conditions for RAPD analysis were consisted of an initial denaturation at 94˚C for 2 min followed by 45 cycles of 60 s at 92 ˚C, 60 s at 36 ˚C and 2 min at 72 ˚C and finally terminated with an extension of 5 min at 72 ˚C in a DNA thermocycler (Bioer. Gene Pro, China). The PCR products were separated in 2% agarose gel

containing 1 μg/ml ethidium bromide, at a constant voltage (60 V) and the number of bands were recorded using a gel documentation system (GENE FLASH).

Results and Discussion

Micropropagated *A. excelsa* R. Br. and their corresponding mother plants produced a total of 2531 bands with an average of 210 bands per primer. The number of loci ranged from 9 in OPA 11 to 23 in OPY 07 with a size ranging from 250 bp in OPH 19 to 3500 bp in OPH 11.OPH 11 produced 17 polymorphic bands but OPH 18, OPX 11 and OPK 17 produced least two polymorphic band. Nearly 68% monomorphic profiles produced in all 12 primers. Indeed, polymorphism information content (PIC) and probability of identity (PI) showed that the OPB 12 was more practicable than other primers in this experiment due to more monomorphic band. In order to know if there is any abnormality in the micropropagated plant, RAPD as a marker that has been revealed to be a potential marker for distinctive genetic variation [1-3], was employed for this purpose, which confirmed that all micropropagated plants had a high affinity to their donor plants. However 8% variation in cultures is might be due to long subculturing and production of adventitious shoot formation. Topophysis is the effect of position of the propagule in the source plant on the phenotype of the progeny plant [33], that it is very important in *A. excelsa* R. Br., plants which are desirable as ornamentals. Very low level of genetic divergence observed in cultured A. excelsa explants, it could be connivance in commercial production even though these low variations exist in seed production of A. excelsa. Further studies with microarray or cDNA AFLP needed to confirm these results. Albeit twelve months old derived plants had an upright stem, their general form was as the same as their seedling with alternative foliage.

Evaluation of genetic fidelity of micropropagated *Araucaria excelsa* R. Br. var. *glauca* using RAPD fingerprints

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Abstract

The aim of this work was to evaluate the micropropagation of *Araucaria excelsa* R. Br. var glauca and finally micropropagated plantlets were evaluated for genetic fidelity using randomly amplified polymorphic DNA (RAPD) fingerprints. Without consideration the effects of explants position, in 0.5 μM 2iP the proliferation rate reached to 4 and mean proliferated shoot's length was 7.65 mm. Explants in the media supplemented with concentrations more than 0.5 μM 2iP and BA if these explants taken from bottom parts of orthotropic stem showed a good proliferation rate; however, their growth was suppressed and finally stopped. Using 0.045 μM TDZ caused 4.60 shoot proliferation rate and 7.08 mm mean shoot length. Results showed a total of 2531 fragments were generated with 12 RAPD primers in micropropagated plants and their donor mother plants. The number of loci ranged from 9 to 23. Cluster analysis of RAPD data using UPGMA (unweighted pair group method with arithmetic average) revealed more than 92% genetic similarities between tissue cultured plants and their corresponding mother plant measured by the Jaccard̕s similarity coefficient.

Similarity matrix and PCoA (two dimensional principal coordinate analysis) resulted in the same affinity. The RAPD showed a high similarity and no differences between mother plant and micropropagated plants derived from them. Primers had shown 32% polymorphism. Subculturing the shoots produced to MS medium containing 7.5 μM IBA and NAA for 15

days before being moved to half strength MS medium without growth regulators resulted in a small percentage (33%) of shoots developed one to two roots per explant. Plantlets were transferred to a mixture of perlite and vermiculite (1:1) under 95% relative humidity and then were transferred to greenhouse condition.

Keyword: Araucaria excelsa, RAPD, genetic fidelity, Micropropagation.