International Journal of Biological Technology (2010) 1(1):94-98

Original Article

Genetic analysis of somoclonal variation among *Jasminum auriculatum* (Vohl.) and it's callus

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Received :15.12.2009; Revised:21.3.2010; Accepted:21.3.2010; Published :15.4.2010

Abstract

In this research article reveals that gentle stability in tissue cultured *Jasminum auriculatum* was examined by random amplified polymorphic DNA (RAPD) analysis. Calli were obtained from *in vitro* grown *Jasminum* leaf segments on MS medium contains 2, 4-D, kinetin and BAP. Calli were induced from explant on MS medium supplemented with 2.5 mg/l of 2,4-D and 0.90 mg/l of KIN,60 to 70 days after calli induction DNA samples from the 10 randomly selected callus and leaf segments of mother plant were subjected to RAPD analysis the detection putative somoclones . A total of 10 arbitrary sequence primers were evaluated, 5 primer produced a high percentage polymorphic amplification products were observed. Mother explant and 2, 4-D induced calli and indicating a high level of genetic variation. Mother explants of *J. auriculatum* showed the smallest somoclonal variation and it compared to the 2, 4-D induced calli having high level of variation this report demonstrates the feasibility of easy induction of regenerative calli by using combination of 2, 4-D and kinetin and the possibility of detecting genetic variation through RAPD analysis among mother and its regenerative calli of *J. auriculatum*.

Keywords: *Jasminum auriculatum*, somoclonal variation, RAPD-PCR.

ABBREVATION: 2,4-D – dicloro phenoxyacetic acid, BAP- benzyl aminpurine , MS – Murashige and Skoog, CTAB – cetyl trimethylammonium bromide.

Introduction

Identification of possible Somaclonal variants at an early stage of development is considered to be very useful for quality control in plant tissue culture, transgenic plant production and in the introduction of variants. Somaclonal variability often arises in tissue culture as a manifestation of epigenetic influence or changes in the genome of differentiating vegetative cells induced by tissue culture conditions (Larkin *et al*., 1981, Muller, *et al*., 1990).

Any genetic change induced by *in vitro* conditions of tissue culture is expected to generate stable plants carrying interesting heritable traits. However, such random changes are not desirable in plant transformation experiments. Therefore, their early detection is considered to be very useful in plant tissue culture and transformation studies. Randomly amplified polymorphic DNA (RAPD) based detection of genetic polymorphism (Welsh,*et al*.,1990, Williams,*et al*.,1990) has found successful application in describing somaclonal variability in regenerated individuals of several plant species (Isshiki, *et al*., 1993, Munthali, *et al.,*1996,Hashmi, *et al* 1997).

Jasminum auriculatum is distributed in the western peninsula of India. It is native of south India and the central provinces. A large number of *j. auriculatum* species are centered on the regions comprising India, China, and Malaysia *(*Khoder *et al.,* 1979)*. J. auriculatum* belongs to the family oleaceae. Climbing shrubs 2 to 3 m in height; branches pubescent. Leaves, shiny auricles, trifoliate; terminal leaf let up to 3.5×1.3 cm, ovate, subglarous, obtusely acute and macronate at apex, rounded at base. The lower leaf let small and frequently. Flowers are white, sweet scented borne in pubescent ,compound many flowered flax cymes ,trichotomous or paniculate cymes ,globose. Corolla lobes are elliptical and fruits black. *(*Muthuswami *et al*., 1972).

Flowers are used for production of perfumes. There is a great demand for jasmine

absolute and concrete in perfume, soap and cosmetic industry, oilmen's and are sold in the market at exorbitant price (Mohamad alikhan *et al.*,1989).

In the present paper, we report successful induction of regenerative calli from *J.auriculatum* leaf explants, cultured on Murashige and Skoog's (MS) medium supplemented with 2,4-D (2,4-dichlorophenoxyacetic acid; as an auxin) and benzylademino purine (BAP; as cytokines), kinetin and the extent of genetic variability in the plants regenerated from one of these calli as examined through RAPD analyses.

Materials and Methods

Jasminum auriculatum Vahl procured from St Xavier's College, Palayamkottai were used in this study. Leaf explants, from a field-grown plant (mother plant), were sequentially washed under running water and with Tween - 20 for 10 min each. Their surface was disinfected with by treated with 0.1% w/v mercuric chloride solution for 3 min. Finally, they were washed 3–4 times with sterile distilled water and inoculated aseptically on MS basal medium (Murashige, T. and Skoog, 1962) containing combinations of 2, 4-D (0.5 to 2.5mg/l) and kinetin (0.1 to 0.9 mg/l). Regeneration of calli was attempted on MS medium containing 2, 4-D (2.5 mg/l) and kinetin (0.9 mg/l). The pH of all media was adjusted to 5.8 and 0.8% weight/volume) agar was added prior to autoclaving at 103 kpa for 20 min.Cultures were incubated under a 12 h photo-period with light intensity of 3000lux at 26 ±1°C.

PCR analysis

For DNA extraction, approximately 1 g fully grown calli was grounded using a hand held grinder with liquid nitrogen and then extracted using cetyl trimethyl ammonium bromide (CTAB) buffer(Doyle and Doyle 1990). The amount of DNA and its quality were assessed by UV spectrophotometer. The DNA is pure enough (OD260/280 =1.68), (Sambrook and Russel, 2000) for RAPD- PCR analysis (Williams *et al*., 1990).

Earlier, ten primers had been tested and five primers which produced reproducible bands and they were selected. The experiments were repeated three times and confirmed the reproducibility of bands. PCR reactions were carried out in a total volume of 20µl at a final concentration of 1 mM $MgCl_2$, 2mM dNTP, *Taq* DNA polymerase enzyme (1u/20µ), with approximately 200 ng DNA as a template and a single random primer (0.2 mM). Conditions were 94°C for 2 min, one cycle, 94°C for 15 sec, 35°C for 15 sec, 72°C for 30sec which were repeated in 40 cycles followed by 5 min-extension at 72°C. Then 8µl of each PCR product was revealed on 1% agarose gel subjected to electrophoresis at 80V after staining using ethidium bromide by UV transilluminator and photographed with gel documentation system Alpha Imager 1200.

Results and Discussion

Previously, a variety of experiments were conducted to select plant growth regulators to establish medium requirements for callus culture in ornamental *Jasminum auriculatum* tissue culture. 2,4-D was effective for inducing callus proliferation in this species. It seemed of interest to screen for the presence of Somaclonal variation in regenerates were obtained in medium supplemented with different 2,4-D concentrations.

High-quality DNA was isolated from the ornamental *J. auriculatum* leaves. Evaluation of Somaclonal variation by RAPD-PCR showed that at least 5 out of 10 primers could reveal some polymorphism in the amplified DNA pattern caused by 2,4-D. The patterns of DNA amplification using different primers are shown in Plate 1-D.

Table -1: Shows the primers, primer sequences, number of bands produced and total number of amplicons revealed by RAPD analysis of its callus.

Primers	Sequences 5'-3'	Total no. of bands	No. of polymorphic bands
OPB05	TGCGCCCTTC	5	-
OPB14	TCCGCTCTGG	6	-
OPB20	GGACCCTTAC	6	1
OPB16	TTTGCCCGGA	5	-
OPB17	AGGGAACGAG	7	1

Table-2: Summary of random amplified polymorphic DNA (RAPD) products from callus and original plant of *J. auriculatum* used in the present study.

Description	RAPD
Number of primers tested	10
Number of primers selected	5
Number of primers that showed polymorphisms	2
Total number of amplified bands	60
Total number of monomorphic amplicons	58
Total number of polymorphic amplicons	2
Percent of polymorphic bands	1.2%
Size of amplified bands	
Average number of polymorphic bands per primer	0.4
Average number of bands per primer	12

Table-3: Presence (+) or absence (-) of polymorphic RAPD bands generated by primers in *J.auriculatum* cultured in media with different 2,4-D concentrations, after 120 days.

S.No.	Polymorphic regenerants	
Primer	Mother plant	Callus
OPB 05	1	-
OPB14	0	-
OPB20	1	1
OPB16	1	0
OPB17	0	1

The 5 primers produced 60 well-defined fragments; among them, 58 bands were monomorphic (98.8%) and 2 bands were polymorphic (1.2%) (Table 2). Genetic variability in propagules of ornamental *Jasminum* of 0.4 bands per primer. The length of the amplification products was between 300 and 1000 bp. The number of bands per primer varied from 5 (OPB-05) to 7 (OPB-17) (Table 1). The polymorphic fragments were produced by 2 primers (OPB-20 and OPB-17). Among the primers that identified polymorphisms, each primers produced one polymorphic band (Table-1). The polymorphism generated by OPB-20 and OBP-17 were observed in single samples with genetic variation (9%). OPB-20 and OBP-17 independently identified one Somaclonal variant (Table-3). Examples of RAPD profile of mother plant and callus are shown in Figure 1-C.

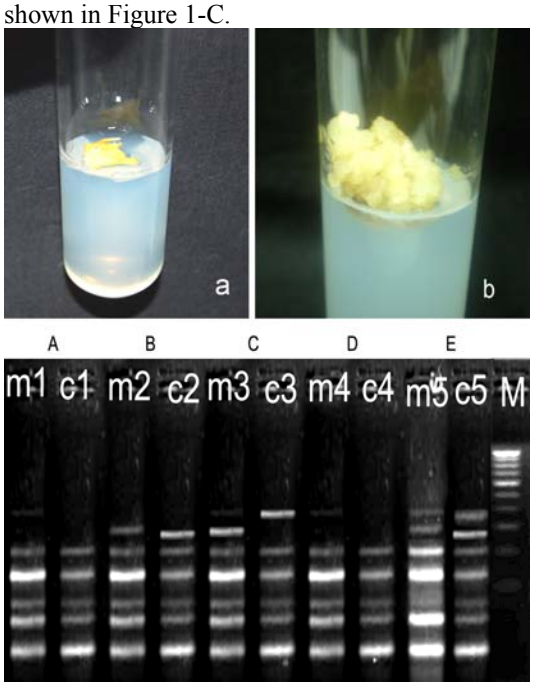

Plate: 1a-c: Molecular pattern of callus revealed by the RAPD with the primers A -OBP-5; B -OBP-14; C- OBP20; D- OPB-16; E -OBP-17; M-markers : m1,m2,m3,m4 and m5 indicates mother plants; c1, c2,c3, c4, and c5 indicate callus

The percentage of polymorphism detected *J. auriculatum* in 2,4-D containing medium was estimated to be 1 to 2 % confirming the occurrence of variation during the callus induction process. The variation of the electrophoretic pattern found in genomic DNA of callus, after 120 days of culture, was probably due to the medium supplementation with 2,4-D (Costa and Zaffari (2005). In *Ananas bracteatus* cv. *striatus*, phenotypic variations of 52% albino plants and 20.5% green plants were described by Costa and Zaffari (2005). In commercial pineapple, Feuser *et al*. (2003) observed a more reduced rate of somaclonal variation among plantlets regenerated from *in vitro* culture using either stationary liquid medium or a temporal immersion system. These authors utilized 10 RAPD primers and detected 7.5 and 5.0% of somaclonal variants for the stationary and temporal immersion systems, respectively. However, in this latter study, the authors did not state the period of culture in which the

samples were taken for RAPD analysis. The culture period directly influences the appearance of somaclonal variation (Skirvin *et al.,* 1994), and the range of variation of 1 to 3% is expected in the process of micropropagation. Some authors have indicated that the treatment used in tissue culture, with high growth rate, may increase the variant numbers (Bairu *et al.,* 2006). The regeneration systems from organized meristems, such as shoot tip and axillary buds, are considered to be the most efficient methods to guarantee genetic integrity of the micropropagated material. The regeneration methods from leave explants (Kawiak and Lojkowska, 2004) and callus (Skirvin *et al.,* 1994) are considered to be less stable permitting the occurrence of genetic variation. In *Drosera binata*, plantlets regenerated through shoot tip preserve the genetic integrity of micropropagated plants. In *Curcuma longa*, rhizome bud explants used to establish cultures show genetic homogeneity in the regenerated propagules, when comparing them with the mother plant (Tyagi *et al*., 2007). However, plants regenerated from leaf base callus have shown variation at the DNA level during *in vitro* culture (Salvi *et al.,* 2001; Tyagi *et al*., 2007).

In this study, RAPD was effective in showing the variations that may occur as a result of mutations during the callus culture of *J. auriculatum* in 2,4-D supplemented medium, and that could be useful in detecting the presence of genetic variation in the initial stages of callus development. In conclusion, the present investigation indicated that mass propagation via tissue cultures produces clones genetically similar to the mother plants. It also conclude that RAPD approach is convenient, fast and reproducible to detect the presence of genetic variation associated with tissue culture of *J .auriculatum* also, these findings could be applied in the breeding of this species.

References

Bairu, M.W., Fennell, C.W. and Van Staden, J. (2006). The effect of plant growth regulators on somaclonal variation in Cavendish banana (*Mus*a AAA cv. '*Zelig*'). *Sci. Hortic.*109: 347-351.

Costa, F. and Zaffari, G.T. (2005). Micropropagação de *Ananas bracteatus* (Shultz) cv. *striatus* Hort. *Rev. Bras. Hort. Ornamental.* 11: 109-113.

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

Feuser, S., Meler, K., Daquinta, M. and Guerra, M.P. (2003). Genotypic fidelity of micropropagated pineapple (*Ananas comosus*) plantlets assessed by isozyme and RAPD markers. *Plant Cell Tissue Organ Cult.* 72:221-227.

Isshiki, S., Okubo, H. and Fuziada, K. (1994). Phylogeny of eggplant and related *Solanum* species constructed by allozyme variation. *Sci. Hort*. 59:171–176.

Karihaloo, J. L. and Gottlieb, L. D. (1995). Random amplified polymorphic variation in the eggplant, *Solanum melongena* L. (Solanaceae).*Theor. Appl. Genet*. 90:767–770.

Kawiak, A. and Lojkowska, E. (2004). Application of RAPD in the determination of genetic fidelity in micropropagated *Drosera* plantlets. *In Vitro Cell. Dev. Biol. Plant.* 40: 592-595.

Larkin, P.J. and Scowcroft, W.R. (1981). Somaclonal variation: a novel source of variability from cell cultures for plant improvement. *Theor. Appl. Genet*. 60: 197-214.

Mohamad Alikhan,W., Raman, V.S and Raman K.R (1989). New chromosomal forms of superior ornamental values in Jasmines. *South Indian hort*. 17:79-83.

Muller, E., Brown, P.T.H., Hartke, S. and Lorz, H.T.I. (1990). DNA variation in tissue-culture-derived rice plants. *Theor Appl Genet*. 80: 673–679.

Munthali, M., Ford-lloyd, B.V. and Newbury, H.J. (1992). The random amplification of polymorphic DNA for fingerprinting of plants. *PCR Methods Appl*. 1: 274-276.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473–497.

Muthuswami, S., Thangaraj, T. and Pappiah, C.M. (1972). Variation in flower characters of J.auriculatum Vahl. seedling . *South Indian Hort.* 20:87-92.

Ramdas, S., Peter, G.B. and Muthuswami, S. (1993) Jasmine In: Bose TK.& Yadav LP (eds) *Commercial Flowers*. Naya Prokash, Calcutta. 486–517.

Salvi, N.D., George, L. and Eapen, S. (2001). Plant regeneration from leaf base callus of

turmeric and random amplified polymorphic DNA analysis of regenerated plants. *Plant Cell Tissue Organ Cult.* 66:113-119.

Sambroog, J . and Russel (2000). Molecular cloning : *A laboratory manual third edition* .Cold Spring Harbour Laboratory press.Newyork:78-85.

Skirvin, R.M., McPheeters, K.D. and Norton, M. (1994). Sources and frequency of somaclonal variation. *HortScience* 29:1232-1237.

Tyagi, R.K., Agrawal, A., Mahalakshmi, C. and Hussain, Z. (2007). Low-cost media for *in vitro* conservation of turmeric (*Curcuma longa* L.) and genetic stability assessment using RAPD markers. *In Vitro Cell Dev. Biol. Plant.* 43:51-58.

Welsh, M. and McCleland, M. (1991). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acid Res*. 18:7213-7218.

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