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Research Article

Assessment of Genetic Fidelity of Micro Propagated Plants of *Aerva lanata* (L.) Juss. ex Schult. using DNA Markers

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Abstract

Aerva lanata (L.) Juss. ex Schult. is a valued medicinal herb. The demand of *A. lanata* is gradually increasing, however the supply is inadequate. Therefore, the need for *in vitro* propagation of this plant is crucial. To check the genetic uniformity and true to true type nature of micro-propagated plants were determined by using DNA markers. Among DNA markers RAPD (random amplified polymorphic DNA) were screened to test the genetic stability of *A. lanata* plants regenerated from nodal segments through direct organogenesis. During the study a total of ten RAPD primers were screened, which produced a total of 32 clear, distinct and monomorphic bands. The micro-propagation protocol developed by our group for rapid in vitro multiplication is suitable for clonal propagation and RAPD marker analysis for genetic fidelity assessment of *A. lanata.*

Keywords: Genetic Fidelity; Aerva lanata; DNA Markers

Introduction

Aerva lanata (L.) Juss. ex. Schult. is an erect dioecious herb, about 80cm in height having cylindrical, branched tap root belonging to Amaranthaceae family. Shoots of this herb are covered with smooth hairs; leaves are simple and alternate, along with obtuse or acute apex. Further the inflorescence consists of axillary spikes and flowers are bisexual, small sessile, greenish white with spikes [37]. This medicinal herb is commonly known as 'Chhaya' in Hindi, 'Bhadram' in Sanskrit, 'Pulai' in Telugu and 'Lopang Ark' in Odia. It has been reported that this plant is used as anti-inflammatory, diuretic, antimicrobial, anticancer, nephroprotective, antidiabetic, antihelimintic, antifertility activities [1-10]. *A. lanata* is loaded with broad range of phyto-constituents. Phytochemical screening showed the presence of various classes of phytochemicals like alkaloids, amino acids and proteins, carbohydrates, saponins, steroids, flavinoids, and tannins [15,16]. The roots possess good amount of aervin, aervoside and methylaervin [17-19], which possibly contribute to its diverse uses in folklore medicines. It has been reported that the whole plant and plant parts including roots and leaves has been used in several herbal preparations to cure various ailments in various pharmaceuticals like Bhadraveradi Kashyam, Dasmolam Kashayam, Nishakathakadi Kashyayam (www. asoka.co.in), Mahasiddhartha oil (www.aparmita.lv). As the raw plant extracts were rich in antioxidants, hence used as tonic by rural poor during pregnancy [11]. At present this important herb is fulfill its demands only from the natural sources. However, wide use of this herb may cause a possible threat for its survivals. Hence *in vitro* culture techniques like organogenesis and somatic embryogenesis may apply for their propagation and conservations of this therapeutic plant [20-21].

Plant tissue culture technique has long been recognized as an efficient tool for rapid clonal multiplication of true to the type. However, the possibility of developing somaclonal variations still exists [23,24] due to several factors such as genotypes used, composition of growth regulators, expression of chromosomal mosaicism, spontaneous mutation, etc. on the other hand, the

occurrence of stable somaclonal variations of specifie type might be advantageous for the crop improvement programme [25,26]. Monitoring the degree of genetic fidelity among micropropagated plants using molecular markers is desirable to reduce the chances for inclusion of variable genotypes and also to assess the technology that is being developed to be marketed. Various molecular markers had been used to assess the genetic fidelity of *in vitro* raised plants by various authers. However, Random Amplified Polymorphic DNA (RAPD) is the cheapest yet reliable and could be a powerful tool for the detection of genetic variability in plants [27-33]. Keeping that entire thing in mind the present objective of this report is assessment of genetic fidelity of micropropagated plants of *A. lanata* using DNA markers.

Materials and Methods

The micro-propagation of *A. lanata* protocol was standardized by Sahu., *et al.* [35], Sahu [36] at Plant Biotechnology Lab, School of Life Sciences, Sambalpur University. For the present study *in vitro* cultures from nodal segments were established on the MS [22] medium fortified with equal conc. BA and kinetin (3.0 mg/l) was found to be the best for direct multiple shoot bud induction, while addition of NAA to the medium with optimum concentration of BA and kinetin fortified MS medium was not found suitable for direct organogenesis. Rather this addition of NAA promotes elongation of shoots and callus mediated organogenesis. For successful rooting of well-developed shoots half strength of MS medium fortified with IBA (0.5mg/L) was used. The survival rate of regenerated plantlets of *A. lanata* was 72 ± 4% [34,35]. The *in vitro* regenerated plants were morphologically alike with the mother plant. For more evidence RAPD banding pattern was compared between mother plant and nine randomly selected *in vitro* regenerated plants by using RAPD primers.

Isolation and purification of genomic DNA

For genomic DNA isolation, about 0.8g of fresh leaves were ground to fine powder in liquid nitrogen and DNA was isolated following the CTAB method [33] with modifications by adding activated charcoal (0.2% v/v) and PVP (3.5% v/v) to the extraction buffer. DNA concentration and purity were measured by using UV–Vis spectrophotometer (UV-1, Thermo, USA) with T10E1 buffer (pH 8.0) as blank. For further confirmation the quantification of DNA was accomplished by analyzing the purified DNA on 0.8% agarose gel along with diluted uncut lambda DNA as standard. All the DNA samples were equilibrated to concentration of 25ng/µl using T10E1 buffer.

RAPD marker analysis

Ten RAPD primers were used for polymorphism detection. List of primers and their information were presented in Table 1. For RAPD analysis PCR amplification of 25 ng of genomic DNA was carried out using decamer oligonucleotide primers (Operon Tech., Alameda, CA, USA). Each amplification reaction of 25µl mixture contained the 25 ng template DNA, 2.5 µl of 10X assay buffer (100 mM Tris.Cl, pH 8.3; 0.5M KCl; 0.1% gelatin), 2.0mM $MgCl_2$, 200 µM each of dNTPs, 20 ng primer, 1.0 units Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). The amplification was carried out in a thermal cycler (Gene Amp-9700, Applied Biosystem, USA) programmed as initial denaturation at 94°C for 5 minutes, cycle denaturation at 94°C for 2 minutes, cycle annealing at 37°C for 1 minute, cycle elongation at 72°C for 2 min for 45 cycles followed by final elongation at 72°C for 5 minutes.

Amplified products were separated on 1.4% agarose gel containing ethidium bromide solution (@ 0.5 µg/ml of gel solutions) in submarine electrophoresis apparatus (Tarson-Midi, India) using TAE (40mM Tris acetate; 2mM EDTA) buffer at constant 50 V for about 1-2 hours. A gel loading buffer (20% Sucrose; 0.1 M EDTA, 1.0% SDS; 0.25% Bromophenol blue; 0.25% Xylene cyanol) was used as tracking dye. Amplified products were visualized by Gel documentation system (Geldoc-XR, Bio-Rad, USA) and photographed. The sizes of the amplified products were determined using 250bp ladder (Bangalore Genei Pvt. Ltd., Bangalore, India) as standard and Quantity One software.

Results and Discussion

Genetic fidelity testing of the micropropagated plantlets needs to be authenticated for commercial scale application of the developed micropropagation protocol. PCR-based molecular markers have emerged as simple, fast, reliable and labor-effective tools for testing the genetic fidelity of *in vitro* raised plantlets. Ten RAPD primers of OPA series (OPA01-OPA10) generated 32 clear amplicons in total (Figure 1). The sizes of the amplified bands were ranging from 400 bp to 2,250 bp. A maximum of six bands was generated from OPA-05 while a minimum of one band was

generated from OPA-07 (Table 1). Monomorphic banding pattern was observed for all the amplified band classes across all the micro propagated plants, along with the donor mother plant with all the tested primers. No polymorphism was observed revealing the genetic integrity of *in vitro* regenerated plants. This method often yields large number of markers; it is technically simple to perform and does not need either large amounts of DNA or previous information of DNA sequences, which are often unknown in many plants. Moreover, RAPD analysis is comparatively easy, cost effective and requires comparatively minimum time and also involves no radioactive compound to be handled. Employing RAPD technique, various authors have reported the absence of genetic variation in many species such as *Zingiber officinales* [27], *Chlorophytum borivilianum* [30], *Picea marina* [31], and *Festuca pratensis* [32], using RAPD analysis. As observed, our result clearly indicates the genetic integrity and true-to-type nature of the *in vitro* regenerated plants in germplasm conservation program.

Primer	Primer sequence (5' - 3')	No. of total bands amplified	Size range (bp)
OPA - 01	5'- CAGGCCCTTC-3'	2	855 -1065
OPA - 02	5'- TGCCGAGCTG-3'	3	400 -2250
OPA - 03	5'- AGTCAGCCAC-3'	2	980 -1275
OPA - 04	5'- AATCGGGCTG-3'	5	655 -1945
OPA - 05	5'- AGGGGTCTTG-3'	6	510 -1645
OPA - 06	5'- GGTCCCTGAC-3'	5	645 - 1700
OPA - 07	5'- GAAACGGGTG-3'	3	525 -1755
OPA - 08	5'- GTGACGTAGG-3'	1	710
OPA - 09	5'- GGGTAACGCC-3'	3	485 - 1125
OPA - 10	5'- GTGATCGCAG-3'	2	1125 -1780

Table 1: List of primers, their sequences, number and size of the amplified fragments generated by random amplified polymorphic DNA (RAPD) primers.

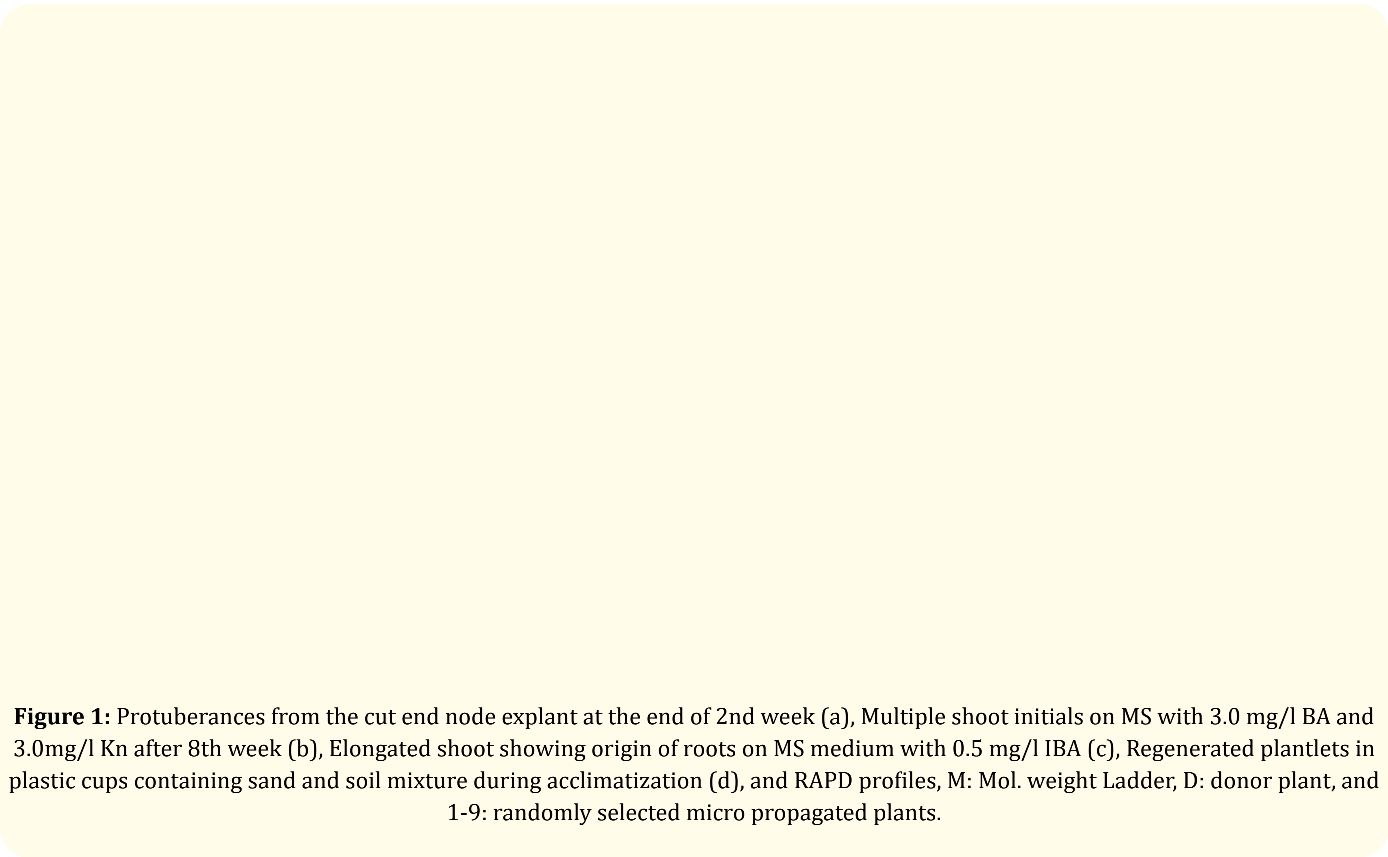

Figure 1: Protuberances from the cut end node explant at the end of 2nd week (a), Multiple shoot initials on MS with 3.0 mg/l BA and 3.0mg/l Kn after 8th week (b), Elongated shoot showing origin of roots on MS medium with 0.5 mg/l IBA (c), Regenerated plantlets in plastic cups containing sand and soil mixture during acclimatization (d), and RAPD profiles, M: Mol. weight Ladder, D: donor plant, and 1-9: randomly selected micro propagated plants.

Conclusion

Aerva lanata (L.) Juss. ex Schult. is a valued medicinal herb. The demand of *A. lanata* is all the time on the increase, however the supply is inadequate. Therefore, the need for in vitro propagation of this plant is crucial. To check the genetic uniformity and true to true type nature of micro-propagated plants were determined by using molecular markers. Among DNA markers RAPD (random amplified polymorphic DNA) were screened to test the genetic stability of *A. lanata* plants regenerated from nodal segments through direct organogenesis. During the study a total of ten RAPD primers were screened, which produced a total of 32 clear, distinct and monomorphic bands. The micro-propagation protocol developed by our group for rapid *in vitro* multiplication is suitable for clonal propagation and RAPD marker analysis for genetic fidelity assessment of *A. lanata.*

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