INTERNATIONAL JOURNAL OF BIOASSAYS
ISSN: 2278-778X
CODEN: IJBNHY
ORIGINAL RESEARCH ARTICLE
OPEN ACCESS

Genetic Fidelity in Micropropagated Plantlets of *Pimpinella tirupatiensis*-an Endemic and Threatened Medicinal Plant Using RAPD and ISSR Markers

Srilakshmi A.[1]*, Ugraiah A.[2], Gayatri M. C.[1] and L. Rajanna[1]
[1]Plant Biotechnology Unit, Bangalore University, Bangalore, Karnataka, India.
[2]Department of Crop Physiology, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India.

Received for publication: March 10, 2016; **Accepted**: March 15, 2016

Abstract: Tissue cultured *Pimpinella tirupatiensis* plantlets were subjected to assessment of genetic stability considering the fact that associated *in vitro* stress might result in breakdown of control mechanism causing instability of the genome. We have used two DNA based molecular markers to assess the genetic fidelity of *in vitro* regenerated *Pimpinella tirupatiensis* through shoot tip from *in vitro* raised seedlings. The shoot tips upon transfer to MS medium containing different concentrations and combinations of 6- benzyl aminopurine (BAP) kinetin (KN), 2- isopentyl adenine (2- ip), α- napthalene acetic acid (NAA) and indole 3- acetic acid (IAA). The best morphogenic response was observed on MS medium fortified with BA (13.31 µM) and NAA (2.69 µM) which exhibited the highest regeneration frequency (90%), the maximum number of shoots/explants (6.50 ± 0.91) and shoot length (3.20 ± 0.20) within 5 weeks. Rooting was achieved within 15 days of shoot implantation on ½ strength MS media fortified with BAP (13.31 µM) and IBA (9.8 µM). The rooted plantlets were successfully acclimatized with 85% survival rate. Out of 20 RAPD and 3 ISSR primers screened, only 6 random amplified polymorphic DNA (RAPD) and all three inter simple sequence repeats (ISSR) primers produced clear reproducible and scorable bands. All banding profiles from micropropagated plants were monomorphic and similar to the mother plant indicating an absence of noticeable genetic variation in the regenerated plantlets. This study is of high significance as these could be commercially utilized for large scale production of true-to-type plantlets in *Pimpinella tirupatiensis*.

Key words: *Pimpinella tirupatiensis*; in vitro propagation; genetic fidelity; RAPD markers; ISSR markers

Introduction

The genus *Pimpinella* L. (Apiaceae) is represented by about 150 species in Europe, Asia and Africa (Mukherjee & Constance 1993). The plants are annual, biannual and perennial usually growing on dry rocky places, rocky crevices, fields, meadows, mountain pastures and grasslands (Bogdanovic & Ruscic 2011). *Pimpinella tirupatiensis* locally known as 'adavikothimeera' (forest coriander) is an herbaceous medicinal plant, distributed on Tirumala hills of Chittor district, Andhra Pradesh (Balakrishnan & Subramanyam 1960, Madhava Chetty *et al.,* 2008). It is a narrow endemic species resembling coriander in certain features and carrot in certain other aspects and is characterized by basal cauline and aerial dimorphic foliage pattern, typical to members of Apiaceae and with subterranean, tuberous root system. Ethnobotanical investigations have showed various medicinal properties, prominent among them are antifertility (Vedavathy *et al.,* 1997) antiulcer and aphtodisiac qualities (Thammanna & Narayana Rao 1990). Over the last few years, extensive damage caused to the natural habitats of rare species of Tirumala hills due to several reasons (Rao *et al.,* 1993) has depleted wild strands of many endemic species in this region. Indiscriminate forest fires, overgrazing, illegal collection of tubers and seeds for various medicinal uses are certain major causes for the rapid depletion of *P. tirupatiensis*. Once described as 'the queen of herbaceous vegetation' of Tirumala Hills (Narayana rao *et al.*, 1983), now the distribution of this rare species is regretfully confined to very few areas. *P. tirupatiensis* is at present under endangered status (Nayar & Sastri 1987). Conventional propagation methods through seed and root tubers for cultivation of *P. tirupatiensis* are best with limited planting material and poor fruit setting. The availability of the seed is also very less due to its dispersal by wind, on attaining maturity. In the present investigation, a regeneration protocol through direct organogenesis is attempted to conserve this rare species of Umbelliferae. *in vitro* multiplication which is an easy and safe method for production of true-to-type plants within a short span of time.

True-to-type clonal fidelity is one of the most important pre-requisites in the micropropagation of plant species. The occurrence of cryptic genetic defects arising via somaclonal variation in the regenerates can seriously limit the broader utility of the micropropagation system (Salvi *et al.,* 2001). It is, therefore, imperative to establish genetic uniformity of micropropagated plants to confirm the quality of the plantlets for its commercial utility. Few reports (Prakash *et al.,* 2001) available so far on *in vitro* multiplication of *P. tirupatiensis* exclude any work on genetic stability analysis of regenerants. Of the various DNA based molecular markers Random Amplified Polymorphic DNA (RAPD) and Inter Simple sequence repeats (ISSR) markers are very simple, fast, cost-effective, highly discriminative and reliable. They require only a small quantity of DNA

***Corresponding Author:**
Srilakshmi Aluri,
Research Scholar,
DST Inspire Fellow, Department of Molecular Biology,
Plant Biotechnology Unit, Bangalore University,
Bangalore- 560 056, Karnataka, India.

sample and they do not need any prior sequence information to design the primer. Thus, they are suitable for the assessment of the genetic fidelity of *in vitro* raised clones. In this study, we assessed the genetic stability of *in vitro* raised *P. tirupatiensis* plants using RAPD and ISSR markers. This technique would facilitate an alternative method for large scale production and successful establishment of genetically stable plants before it is released for commercial purpose.

Material and Methods

Collection of Plant Material and Explants Preparation

Mother plants of *P. tirupatiensis* were collected from Tirumala hills (1000m above msl), Andhra Pradesh, India and authenticated by Dr. S. Rajan, field botanist at CSMPC, Emerald, Ooty. The collected plants were established in the Molecular Biology medicinal plant garden, Bangalore University, Bangalore. India. The explants shoot tips were collected from *in vitro* grown seedlings of *P. tirupatiensis* under aseptic conditions to initiate shoot multiplication.

Media and Culture Conditions for Shoot Multiplication

Under aseptic conditions, the explants were inoculated on MSBM (Murashige and Skoog 1962) supplemented with different concentrations and combinations of BAP (2.22 µM, 4.44 µM, 8.87 µM,13.31 µM and 22.19 µM), Kn (2.32 µM, 4.6 µM, 9.29 µM, 13.94 µM and 23.2 µM), 2 ip (2.46 µM, 4.92 µM, 9.84 µM, 14.76 µM and 24.61 µM), NAA (2.69 µM, 5.37 µM and 8.06 µM) and IAA (2.85 µM, 5.71 µM and 8.56 µM) individually and in combinations to study their response on shoot bud multiplication. The pH of the medium was adjusted to 5.8 prior to the addition of 0.8% agar and autoclaved at 121°C, 15 lbs. pressure for 15 min. All the cultures were incubated at 25 ± 2°C under a 16 h light and 8 h dark regime with a light intensity of 3,000 lux provided by cool-white fluorescent tubes. Data with respect to percent shoot multiplication, number of shoots/explants, and shoot length per culture were recorded after 30 days of subculture.

In vitro Rooting and Acclimatization

After multiplication, regenerated shoots were separated and transferred to the rooting medium. The regenerated shoots (2-3 cm) were excised and cultured on freshly prepared rooting medium containing half-strength MS medium fortified with BAP (13.31 µM) and IBA (9.8 µM). After 4 weeks of culture, the frequency of root formation, number of roots produced per cultured shoot, and length of the root were recorded. Plantlets with well-formed roots were removed from culture medium, washed gently in running tap water and transferred to pots containing vermiculite soil and sand, (1:1). These plantlets were covered with a polythene cover ensuring high humidity (85%) and watered for every 3 - 4 days with quarter-strength MS salt solution without sucrose and vitamins. After 20 days, the polythene cover was removed and maintained in pots and subsequently transferred to the field.

Molecular Characterization of Regenerated *P. tirupatiensis* Plants

DNA was isolated from leaves of randomly selected hardened *in vitro* plants after 3 months of their transfer to field conditions and DNA was also extracted from the *in vivo* mother plant using the modified cetyltrimethyl ammonium bromide (CTAB) method described by Reineke *et al,* (1998). The quality of the extracted DNA was verified by sample electrophoresis on 0.8% (w/v) agarose gels prepared in 1X TAE buffer. Aliquots of 1000 bp uncut lambda DNA ladder (100 ng µL-1) were used as standards. Ethidium bromide stain (1 mg L-1) was used to visualize the DNA. The concentration of DNA was measured by spectrophotometric analysis. The RAPD and ISSR analysis was performed following the methodology of Williams *et al.,* (1990). Each amplification of 25 µl consisted of 10 µl of DNA (20ng), 2.5 µl 10X assay buffer (100 mM Tris-Cl, pH 8.3, 0.5M KCl, 1.5 mM MgCl2 and 0.01% gelatin), 0.5 µl of dNTP mix, 1 µl primer (20pm) and 1 µl of 1 U Taq DNA polymerase (Bangalore Genei Pvt. Ltd, Bangalore, India) and 10 µl of sterile double distilled water. RAPD - PCR amplification was carried out in 25 µL volume using six different decamer primers (Operon Technologies Inc., USA). ISSR- PCR amplification was carried out in 25 µL volume using three different Inter Simple Sequence Repeat primers. The amplification was carried out in a thermal cycler (GeneAmp PCR System 9700, Applied Biosystem, USA). The first step consisted of complete denaturation of the sample template DNA at 94ºC for 5 minutes. The second step consisted of 42 cycles, each cycle with three temperature ranges: denaturation of template DNA at 92ºC for 1 minute, primer annealing at 45-54ºC for 1min and primer extension at 72ºC for 2 min. followed by complete polymerization of the sample at 72ºC for 7 min. The soaking temperature was 4ºC. After completion of the PCR, 2.5 µl of 6X loading dye (MBI Fermentas, Lithuania) was added to the amplified product, which was deep frozen at –20ºC for future use. The amplicons were separated in 1.5 and 2 % agarose gel for RAPD and ISSR respectively. Electrophoresis was performed at a constant voltage at 60 V for 3 hours. The amplicons were visualized under UV light and photographed. The gel was documented by Gel Doc 2000 (Bio-Rad, USA) for scoring the bands. The amplicon size was determined by comparison with the ladder.

Statistical Analysis

The experiments were set up in completely randomized design. Each culture tube with one shoot explant was considered as one replicate. Each treatment in each set of experiments consists of 15

replicates and each experiment was repeated three times. Standard error of means was calculated in each experiment. The data was statistically analyzed using one-way analysis of variance (ANOVA), and means were compared using the DMR test at the 0.05% level.

Results and Discussion

Shoot Multiplication, *in vitro* Rooting of Plantlets and Acclimatization

The effects of different concentrations and combinations of cytokinins and auxins and on morphogenetic response of explants after 30 days of culture were explored and summarized in Table 1 & 2. Direct organogenesis was exhibited by explants on MS medium containing different concentrations of BAP (2.22 µM, 4.44 µM, 8.87 µM, 13.31 µM and 22.19 µM), Kn (2.32 µM, 4.6 µM, 9.29 µM, 13.94 µM and 23.2 µM), 2 ip (2.46 µM, 4.92 µM, 9.84 µM, 14.76 µM and 24.61 µM), NAA (2.69 µM, 5.37 µM and 8.06 µM) and IAA (2.85 µM, 5.71 µM and 8.56 µM) individually and in combinations. Shoot regeneration frequency ranging from 40 to 82% was obtained in all the treatments. It was noticed in the present study that the culture responded differently in different treatments. However, the highest mean number of shoots 4.50 were observed on MSBM supplemented with BAP (13.31 µM) and the lowest mean number 1.00 on MSBM fortified with Kn (2.32 µM) (Table 1). Further, an increase in the mean number of shoots from 4.50 to 7.50 was observed on BAP (13.31 µM) and NAA (2.69 µM) (Table 2).

Table 1: Effect of various concentrations of BAP, Kn and 2iP with MSBM on shoot tip explant of *Pimpinella tirupatiensis*

Growth regulators (µM/l)	Shoot sprouting frequency (%)	Mean shoot no. / explant±SE*	Mean length of shoots (cm) ±SE*
BAP			
2.22	45	1.03 ± 0.09 ^{d}	2.00 ± 0.04 ^{c}
4.44	66	1.90 ± 0.21 ^{bc}	2.86 ± 0.49 ^{c}
8.87	77	3.93 ± 0.12 ^{b}	2.66 ± 0.04 ^{a}
13.31	82	4.30 ± 0.17 ^{a}	2.53 ± 0.23^{a}
22.19	46	1.16 ± 0.12 ^{bd}	2.36 ± 0.20 ^{b}
Kn			
2.32	40	1.42± 0.14 ^{d}	1.60± 0.15 ^{d}
4.65	48	1.50 ± 0.00 ^{b}	2.06 ± 0.41 ^{b}
9.29	60	1.96 ± 0.12 ^{a}	3.73 ± 0.04 ^{a}
13.94	53	1.66 ± 0.17 ^{b}	2.16 ± 0.33 ^{c}
23.20	26	1.06 ± 0.24 ^{b}	2.00 ± 0.37 ^{cd}
2-iP			
2.46	48	1.76 ± 0.18 ^{d}	1.76 ± 0.12 ^{bc}
4.92	55	2.33 ± 0.36 ^{b}	2.13 ± 0.33 ^{a}
9.84	64	2.43 ± 0.09 ^{ab}	1.80 ± 0.08 ^{b}
14.76	72	3.23 ± 0.12 ^{c}	1.60 ± 0.16 ^{d}
24.61	57	2.70 ± 0.21 ^{c}	1.33 ± 0.18 ^{de}

*Means ± SE, n = 45. Means followed by the same letter are not significantly different by the DMR test at 0.05% probability level

Shoot tips are ideal vegetative propagules for large scale production through *in vitro* micropropagation as they could provide similar plants with desired characters as reported by Bajaj *et al.,* (1977) and George (1993). MSBM fortified with BAP (13.31 µM) and NAA (2.69 µM) was found to be the suitable medium for shoot tip initiation and multiplication (Figs 1, 2, 3 & 4). This combination is significantly superior when compare to other treatments with respect to multiple shoot formation in *P. tirupatiensis* using axenic shoot tips. This does not agree with the findings of Goleniowski *et al.,* (2003). They have reported that MSBM supplemented with low concentration of BAP and NAA are suitable for *Oreganum vulgare* (Apiaceae member) shoot multiplication. Lower concentration of BAP will not enhance the multiplication in *P. tirupatiensis*. Further, it was found in the present investigation that high concentration of BAP and low concentration of NAA promote shoot formation in *P. tirupatiensis*.

Table 2: Effect of different combinations of BAP, KN, NAA and IAA with MSBM on shoot tip explant of *Pimpinella tirupatiensis*

Growth regulators (µM/l)	Shoot sprouting frequency (%)	Mean shoot no./ explant±SE *	Mean length of shoots (cm) ±SE *
BAP+ NAA			
13.31+2.69	90	6.50 ± 0.91 ^{a}	3.20 ± 0.20 ^{a}
13.31+5.37	74	5.43 ± 0.36 ^{b}	2.13 ± 0.33 ^{bc}
13.31+8.06	65	3.93 ± 0.09 ^{d}	1.80 ± 0.08 ^{c}
BAP+IAA			
13.31+2.85	63	3.99 ± 0.19 ^{d}	1.70 ± 0.18 ^{c}
13.31+5.71	59	4.00 ± 0.21 ^{cd}	1.56 ± 0.24 ^{c}
13.31+8.56	55	4.64 ± 0.21 ^{c}	1.16 ± 0.24 ^{c}
KN+ NAA			
9.29+2.69	55	4.63 ± 0.21 ^{c}	1.26 ± 0.24 ^{c}
9.29+5.37	75	5.43 ± 0.36 ^{b}	2.13 ± 0.33 ^{b}
9.29+8.06	63	4.23 ± 0.27 ^{c}	2.50 ± 0.21 ^{b}
KN+ IAA			
9.29+2.85	70	4.89 ± 0.30 ^{c}	2.90 ± 0.31 ^{ab}
9.29+5.71	65	4.43 ± 0.27 ^{c}	2.70 ± 0.21 ^{b}
9.29+8.56	58	3.26 ± 0.00 ^{d}	1.93 ± 0.27 ^{c}

*Means ± SE, n = 45. Means followed by the same letter are not significantly different by the DMR test at 0.05% probability level

Higher concentration of BAP enhances the multiplication and elongation of shoots. This coincides with the findings of Segura *et al.,* (1987). They have opined that to achieve high frequency of shoot initiation it is essential to supply cytokinins and auxins to the culture media. The highest frequency of root formation, maximum number of roots and root length were achieved on half-strength MS medium supplemented with BAP (13.31 µM) and IBA (9.8 µM). After 4 weeks of culture, 100 % of root formation, 5-10 roots produced per cultured shoot, and length of 4-5 cm root were recorded (Fig. 5). Plantlets with well-formed roots were removed from culture medium, washed gently in running tap water, and transferred to pots containing vermiculite soil and sand, (1: 1). These plantlets were covered with a polythene cover ensuring high humidity (80%) and watered for every 3-4 days with quarter-strength MS salt solution without sucrose and vitamins. After 20 days, the polythene cover was removed and maintained in pots, and subsequently hardened with 85 % survival rate (Fig.6)

Table 3: Number of amplification products generated with the use of RAPD primers for the genetic analysis of *P. tirupatiensis*

S. No	Primer	Sequence	No. of bands
1	OPAF-03	AGTCAGCCAC	3
2	OPAF-05	CCCGATCAGA	4
3	OPAF-06	CCGCAGTCTG	8
4	OPAF-15	CACGAACCTC	6
5	OPC-16	CAGCGACTGT	5
6	OPN-16	AAGCGACCTG	2

Table 4: Number of amplification products generated with the use of ISSR primers for the genetic analysis of *P. tirupatiensis*

S. No	Primer	Sequence	No. of bands
1	(GACA)4	(GACA)4	5
2	(AGG)6	(AGG)6	3
3	T(GA)9	T(GA)9	3

Figs. 1-6: ***In vitro*** **propagation of** ***Pimpinella tirupatiensis:*** **1. Shoot tip of** ***P. tirupatiensis*** **measuring 1 cm at culture 2. Tiny leaves after 15 days of culture 3. Shoots after 35 days of culture 4. Multiple shoots after 60 days of culture 5. Shoots with roots after 60 days of culture 6. Hardened plant**

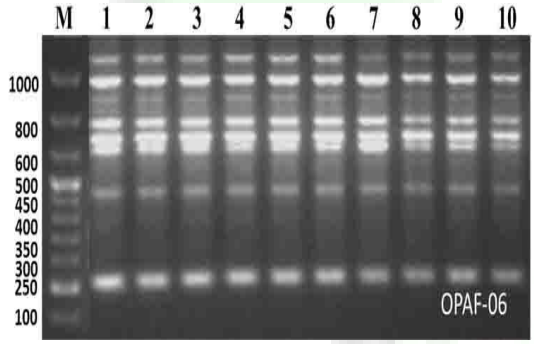

Fig. 7 Ethidium bromide stained agarose gel showing amplification pattern of mother plant, 2-9 showing amplification pattern of randomly selected *in vitro* raised plantlets using random primer OPAF- 06. M.1000 bp DNA ladder

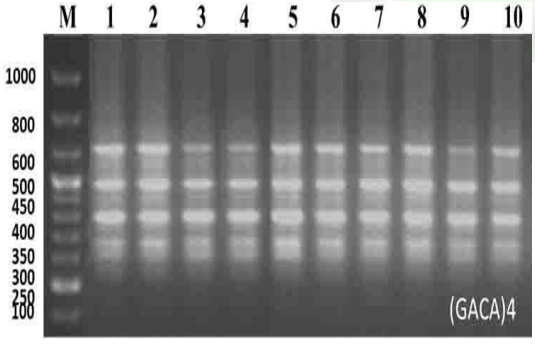

Fig. 8 Ethidium bromide stained agarose gel showing amplification pattern of mother plant, 2-9 showing amplification pattern of randomly selected *in vitro* raised plantlets using ISSR primer (GACA)4. M.1000 bp DNA ladder

RAPD and ISSR Analysis of Regenerated Plants

Micropropagation is used to obtain uniform planting material. However, it is necessary to authenticate the clonal fidelity of *in vitro* regenerated plants to confirm the reliability on the protocol for mass multiplication. Taylor *et al.,* (1995) and Choudhuri *et al.,* (2009) reported that PCR-based techniques such as RAPD and ISSR markers were successfully employed for the detection of variations at the genome level among regenerants. Since RAPD and ISSR techniques are simple and cost effective, it has been widely used by many researchers to assess genetic stability in micropropagated plants (Agnihotri *et al.,* 2009; Chalageri and Babu 2012; Paul *et al.,* 2010; Sun *et al.,* 2009). Hence, in the present study we employed RAPD and ISSR analysis to check the genetic uniformity among the micropropagated plants and mother plant. For RAPD analysis out of six primers (Table 3), the best amplified primer (OPAF-06) was selected which produced a total of eight scorable bands ranging from 100 to 1000 bp all of which were found to be monomorphic in nature (Fig.7). For ISSR analysis out of three primers (Table 4), the best amplified primer (GACA)4 was selected, which produced a total of five scorable bands ranging from 100 to 1000 bp all of which were found to be monomorphic in nature (Fig.8). The results of the RAPD and ISSR analysis showed no evidence of polymorphisms or changes between the micropropagated plants and mother plants of *P. tirupatiensis* suggesting the genetic stability among the plants.

Conclusion

From the present study, it has been concluded that RAPD and ISSR analysis can be used to check the genetic consistencies in the micropropagated plants and donor plant of *P. tirupatiensis.* This medicinal plant species requires immediate attention with greater emphasis for habitat protection and incorporation of larger germplasm collection and other propagation techniques. *In vitro* plant regeneration and reintroduction of the threatened plants into the original or favorable habitats is one strategy for conservation of important medicinal plant species. The observation of monomorphism between regenerants supports the conclusion that these plantlets regenerated by the method used in this study are likely to be genetically true to their plant of origin.

Acknowledgement

The author Srilakshmi A is gratefully acknowledged to DST – INSPIRE program for providing financial support to undertake this program.

References

1. Agnihotri RK, Mishra J and Nandi SK. "Improved *in vitro* shoot multiplication and rooting of Dendrocalamus hamiltonii Nees et Arn. Ex Munro: production of genetically uniform plants and field evaluation". *Acta Physiol. Plant* 31: (2009): 961-967.

2. Bajaj, YPS. "Protoplast isolation, culture and somatic hybridization". In: Reinert, J. and Bajaj Y.P.S (Eds.) Applied and fundamental aspects of plants cell tissue and organ culture, Springer-verlag, New York. (1977): 449-467.

3. Balakrishnan, N. P and Subramanyanm. "A new species of Pimpinella from Tirupathi hills, South India". *Bull Bot. Surv. India.* 2: (1960): 427 – 428.

4. Bogdanovic, S. and Ruscic, M. "Pimpinella tragium Vill. subsp. Lithophila (Schischk.) Tutin (Apiaceae), a new taxon in Croatian flora". *Acta. Bot.Croat,* 70.1: (2011): 115-120.

5. Chalageri G and Babu UV. "*In vitro* plant regeneration via petiole callus of Viola patrinii and genetic fidelity assessment using RAPD markers". *Turk. J. Bot.* 36: (2012): 358-368.

6. Choudhuri, R.K., Pal, A. And Jha, T.B. "Regeneration and characterization of Swertia chirata Buch.-Ham. ex Wall. plants from immature seed cultures". *Scientia Hort.* 120: (2009): 107-114.

7. George EF In: George, E.F. (Ed.), "Plant propagation by tissue culture". Part 2: In practice, Exergetics ltd., Edington, England. (1996).

8. Goleniowski ME, Flamaraque, C. and Bima P. "Micropropagation of Origano (Origano vulgar x Aplii) from meristem tips*". In vitro Cell Dev. Biol- Plant* 39: (2003): 125-128.

9. Madhava Chetty K, Sivaji K & Tulasi Rao K "Flowering plants of Chittor district Andhra Pradesh India" Scientific Publishers, Jodhpur India. (2008): 330-333.

10. Mukherjee, P. K. and Constance. "Umbelliferae (apiaceae) of lndia", American institute of indian studies & oxford & ibh publishing co. New delhi. (1993): 137-155.

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

12. Narayana Rao, K. and Raja Reddy, K., in Proc. Nat. Sem. (eds Jain, S. K. and Rao, R. R.), at Dehradun, 14–17 September 1981, BSI, India, (1983): 157–163.

13. Nayar, M. P. and Sastry, A. R. K., "Red Data Book of Indian Plants", Calcutta Botanical Survey of India, 1: (1987): 367.

14. Paul A, Thapa G, Basu A, Mazumdar P, Kalita MC and Sahoo L. "Rapid plant regeneration, analysis of genetic fidelity and essential aromatic oil content of micropropagated plants of Patchouli, *Pogostemon cablin* (Blanco) Benth. – An industrially important aromatic plant". *Ind. Crops Prod.* 32.3: (2010): 366-374.

15. Prakash, E. "*In vitro* studies on *Pimpinella tirupatiensis* Bal. & Subr. An endemic medicinal plant". Ph. D. Thesis submitted to Sri Venkateswara University, Tirupathi, India. (2001).

16. Rao, K. N., Chetty, K. M. and Sandhya Rani, Indian Bot. Rep., 12: (1993): 11–16.

17. Reineke AP and Zebitz CPW. "Preparation and purification of DNA from insects for AFLP analysis", *Insect Mol. Biol* 7: (1998): 95-99.

18. Salvi ND, George L, Eapen S. "Plant regeneration from leaf base callus of turmeric and random amplified polymorphic DNA analysis of regenerated plants". *Plant Cell Tissue and Organ Culture* 66. 1 (2001) :13–119.

19. Segura J and Calvo MC. Lavandula spp. (Lavander): "In vitro culture, regeneration of plant and the formation of essential oil and pigments". Biotech. in Agri and Forestry, 15 medicinal and aromatic plants III (ed. by Y.P.S. Bajaj) 15: (1987): 283- 310.

20. Sun Y, ZhaoY, Wang X, Qiao G, Chen G, Yang Y, Zhou J, Jin L and Zhuo R. "Adventitious bud regeneration from leaf explants of *Platanus occidentalis* L. and genetic stability assessment". *Acta Physiol. Plant* 31: (2009): 33-41.

21. Taylor, P.W.J., Geijskes, H.L., Fraser, T.A. Henry, R.J. And Birch, R.G. "Sensitivity of random amplified polymorphic DNA analysis to detect genetic change in sugar cane during tissue culture". *Theor. Appl. Genet.* 90: (1995): 1169-1173.

22. Thammanna & Narayana Rao K. "Medicinal Plants of Tirumala" TTD Press Tirupati. (1990): 36.

23. Vedavathi S, Mrudula V & Sudhakar A. "Tribal Medicine of Chittoor District AP" Herbal Folklore Research Centre, Tirupati. (1997): 110 - 111.

24. Williams, J G K, kubelic, A R, Livak, K. J, Rafalski, J A and Tingey S V. "DNA polymorphisms amplified by arbitrary primers are useful as genetic markers". *Nucl. Acid Res* 18: (1990): 6531-6653.

Cite this article as:
A. Srilakshmi, A. Ugraiah, M. C. Gayatri and L. Rajanna. Genetic Fidelity in Micropropagated Plantlets of *Pimpinella tirupatiensis*-an Endemic and Threatened Medicinal Plant Using RAPD and ISSR Markers. *International Journal of Bioassays* 5.6 (2016): 4661-4666.

Source of support: Nil
Conflict of interest: None Declared