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Full Length Research paper

Genetic variability among *Coleus* sp. studied by RAPD banding pattern analysis

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Genetic improvement of medicinal plants depend on the existence, nature and extent of the genetic variabiles available for manipulation. Genetic analysis with random amplification of polymorphic DNA markers has been extensively used to determine genetic diversity among *Coleus* sp. and to identify the best quality for human consumption and its medicinal purpose. The objectives of the present study were to assess molecular variation among *Coleus amboinicus, Coleus aromaticus* and *Coleus forskohlii* and to determine the level of genetic similarity among them. Analyses carried out include random amplification of polymorphic DNA (RAPD) on three strains of *Coleus* sp. and random primers were used for PCR. Electrophoresis on denaturing acrylamide gels improved RAPD reproducibility and increased the band number. In this study, the primer OPW 6 and OPW 7 gave reproducible results and the band profiles.

Key words: RAPD, genetic diversity, conservation, primers, genetic polymorphism.

INTRODUCTION

Medicinal plants play a vital role to preserve our health. The genus, *Coleus* consists of herbs, that are widespread in all over India and represents highly valuable plant species having therapeutic and nutraceutical importance. Genetic variation is essential for long term survival of species and it is a critical feature in conservation. For efficient conservation and management, the genetic composition of the species in different geographic locations needs to be assessed. In recent years, fingerprinting systems based on RAPD analysis have been increasingly utilized for detecting genetic polymorphism in several plant genera.

Coleus sp. is one of the important medicinal plants extensively used by traditional practiceoners in India for its medicinal value. *Coleus forskohlii* has been traditionally used to treat high blood pressure. Other benefits include help in losing weight, improving digestion and nutrient absorption, fighting cancer, and immune system support (Gilbert et al., 1999). RAPD analysis (Williams et al., 1990; Welsh and McClelland, 1990) is capable of detecting differences among strains of a single species. The simplicity and fast sample processing of RAPD technique makes it useful for assessing population genetic parameters such as within-population and between-population genetic diversity. An additional advantage is that knowledge of the DNA sequences is not necessary to apply this technique (for a review on this technique see Weising et al., 1995). PCR based RAPD markers have been widely used in assessing genetic variation with in a species by measuring genetic diversity in many species, including medicinal plants (Rosa et al., 2005).

Since variability is a prerequisite for selection programme, it is necessary to detect and document the amount of variation existing within and between populations. DNA marker based fingerprinting can distinguish species rapidly using small amounts of DNA and therefore can assist to deduce reliable information on their phylogenetic relationships. DNA markers are not typically influenced by environmental conditions and

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Table 1. Primers used in RAPD-PCR reactions.

S. No	Primer	Sequence (5'-3')
1	OPW 6	ACGCCCGATG
2	OPW 7	CTGGACGTCA
3	OPW 8	GACTGCCTCT
4	OPW 9	GTGACCGAGT
5	OPW 10	TCGCATCCCT
6	OPU 15	ACGGGCCAGT
7	OPU 16	CTGCGCTGGA
8	OPU 17	ACCTGGGGAG
9	OPU 18	GAGGTCCACA
10	OPU 19	GTCAGTGCGG

therefore can be used to describe patterns of genetic variation among plant populations and to identify duplicated accessions within germplasm collections (Mohamad et al., 2009).

Molecular markers have been shown to be useful for genetic variation of plant species. These markers based on the polymerase chain reaction (PCR) technique which is the most commonly used for these purposes. Several different PCR- based techniques have been developed during the last decade, each with specific advantages and disadvantages. The randomly amplified polymorphic DNA (RAPD) markers techniques is quick, easy and requires no prior sequence information; it detects nucleotide sequence polymorphisms using single primer of arbitrary nucleotide sequence (Williams et al., 1990). RAPD marker have been extensively used for DNA fingerprinting (Moreno et al., 1998) (Gilbert, 2001), (Gilbert et al., 1999*)* and (Gilbert et al., 2006), genetic diversity studies (Hoz et al., 1996).Population genetic studies (Wolfe et al., 1998; Wu, 2005).

Among the different types of molecular markers available, random amplified polymorphic DNA (RAPD) are useful for the assessment of genetic diversity because of their simplicity, speed and relatively low cost compared to other molecular markers (William et al., 1990; Rafalski and Tingey, 1993).in this we identify the genetic variability of *Coleus* sp. medicinal plants.

MATERIALS AND METHODS

Plant sample

Medicinal plants such as *Coleus amboinicus, Coleus aromticus and C. forskohlii* were collected from botanical garden of Kollihills, Namakkal, Tamilnadu (India).

DNA extraction

Two leaves of 2-week-old seedlings were ground in a mortar with liquid nitrogen. Then 1.5 ml of extraction buffer (Tris-HCl 100 mM, pH 7; EDTA 100 mM, pH 7; NaCl 3 M) and SDS at a final concentration of 1% were added. Each sample was shaken and 1.5 ml of equilibrated phenol was added. The phases were separated by centrifugation at 13,000 rpm for 30 min; 500 ml of supernatant was transferred to 1.5 ml eppendorfs and 2 volume of 100% ethanol and 50 ml of 3 M sodium acetate were added to the supernatant for DNA precipitation. The pellets were washed with 70% ethanol and re-suspended in 200 ml of Tris EDTA. Samples were treated with RNAse, and the DNA purity was estimated by measuring the OD (optical density) at 260 to 280 nm (Martins et al., 2006).

RAPD analysis

The random primers (OPW 6-10 and OPU 15-19) were purchased from chromous Bsiotech, Bangalore, India (Table 1). RAPD reactions were carried out in a 20 µl reaction volumes containing 1 µl of template DNA solution (about 50 mg), 0.25 µM primers, 100 µM dATP, dGTP, dCTP, dTTP, respectively, 2.5 mM Mg^{2+}, 1X polymerase buffer and 1.0 U Taq DNA polymerase (Chromous Biotech Bangalore). PCR amplification reaction was conducted with Masteral Thermal Cycler (Corbett Research Australia). The cycling parameters were 94°C, 5 min and 45 cycles to denature at 94°C, 30 s; anneal at 36°C, 1 min; extend at 72°C, 2 min. the amplification products were separated on the 1.2% agarose gel for 1 to 2 h at 80 V, and recorded with the Alpha digital imager gel documentation system (Alpha innotech, Australia) after staining with ethidium bromide (0.5 µg/ml). All tests were repeated twice.

Electrophoresis and silver stain

The RAPD-PCR reactions were electrophoresed in a 12% horizontal acrylamide gel (0.5 mm) refrigerated at 15°C (200 V/ 23 mA/5 W for 10 min followed by 600 V/30 mA/18 W for 2 h). Lambda Marker was used to obtain the band sizes. The bands were visualized by silver staining with a Silver Xpress kit (Bio-Rad), based on fixing with acetic acid, silvering with $AgNO_3$, and developing with Na_2CO_3-sodium thiosulphate. The gel was finally impregnated with acetic acid-glycerol and air-dried.

Quantitative analysis of DNA

The purity and quantity of the isolated DNA was determined by Nanodrop spectrophotometry. The extinction ratio (260/280 nm) was found between 1.4 to 1.8 that indicated the DNA was pure enough for RAPD analysis.

Table 2. Total number of bands.

S No.	Samples	Operon code					Total
		OPW 6	OPW 7	OPW 8	OPW 9	OPW 10	
1.	*Coleus amboinicus*	8	7	0	0	0	15
2.	*Coleus aromaticus*	0	0	0	0	0	0
3.	*Coleus forskohlii*	2	0	4	4	3	13
							28

S No.	Samples	Operon code				
		OPU 15	OPU 16	OPU 17	OPU 18	OPU 19
1.	*Coleus amboinicus*					
2.	*Coleus aromaticus*			NO BANDS		
3.	*Coleus forskohlii*					

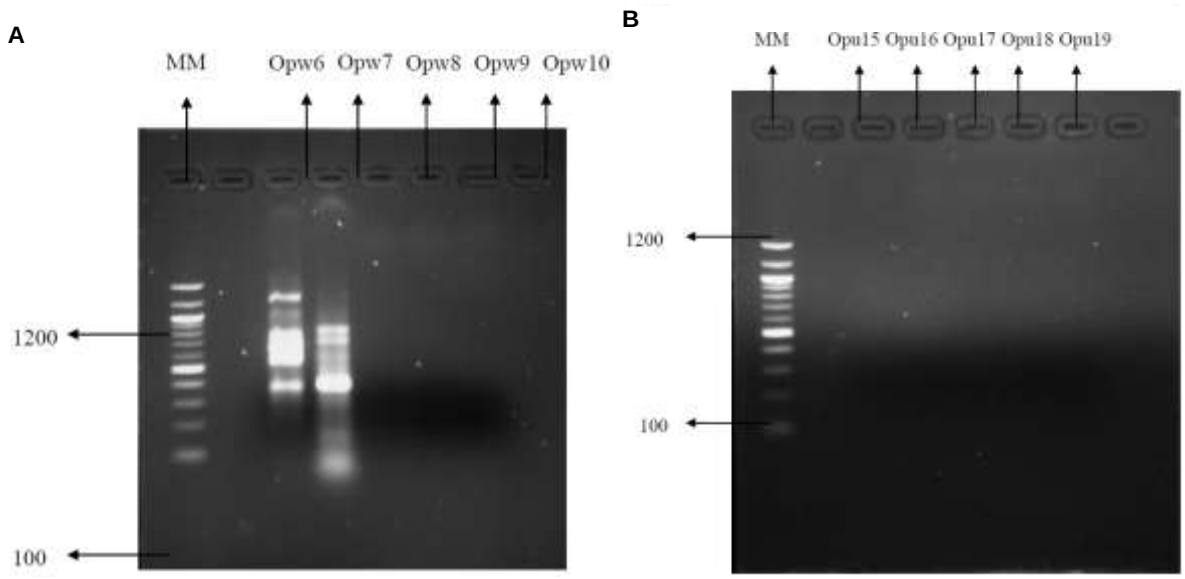

Figure 1. RAPD-PCR products of *Coleus amboinicus.*

RESULTS AND DISCUSSION

The RAPD technique was used to find out the extent of genetic diversity in *Coleus* sp. and primers OPW (6-10) and OPU were used for amplification. Maximum number of bands were observed in *C. amboinicus w*ith primer OPW 6 followed by OPW 7 (Table 2). A sum total of 28 bands were amplified with respect to all the 5 primers (OPW 6-10). By using OPW (6-10) total number of bands amplified were 28, where as there was no amplification in OPU (15-19).So that variation among *Coleus* sp. was identified from OPW but not in OPU primers. In *C. amboinicus* totally 15 bands were amplified, in *C. aromaticus* there was no amplification, *C. forskohlii* 13 bands were amplified.

The maximum numbers of bands were identified from *C. amboinicus* collected from botanical garden of Gandhi Krushi Vignana Kendra (GKVK) and Biocentre in Bangalore, followed by the *C. forskohlii*. The bands obtained ranged in size from 100 - 1200 bp. In the present study genetic variability among the three *Coleus* sp. were determined by RAPD technique by using the random primers OPW (6-10) and OPU (15-19) series. The PCR products obtained were analyzed (Figures 1a, b, 2a, b, 3a, and b).

The purity and quantity of the isolated DNA was determined by Nanodrop spectrophotometry. The extinction ratio (260/280 nm) was found between 1.4 to 1.8 that indicated the DNA was pure enough for RAPD analysis (Figure 4a, b, c). Phylogenetic variation were determined in *Coleus* sp. by converting RAPD data into a frequency similarity and analysed by Unweighted Pair Group Method with Arithmetic mean (UPGMA) (Figure 5 and 6).

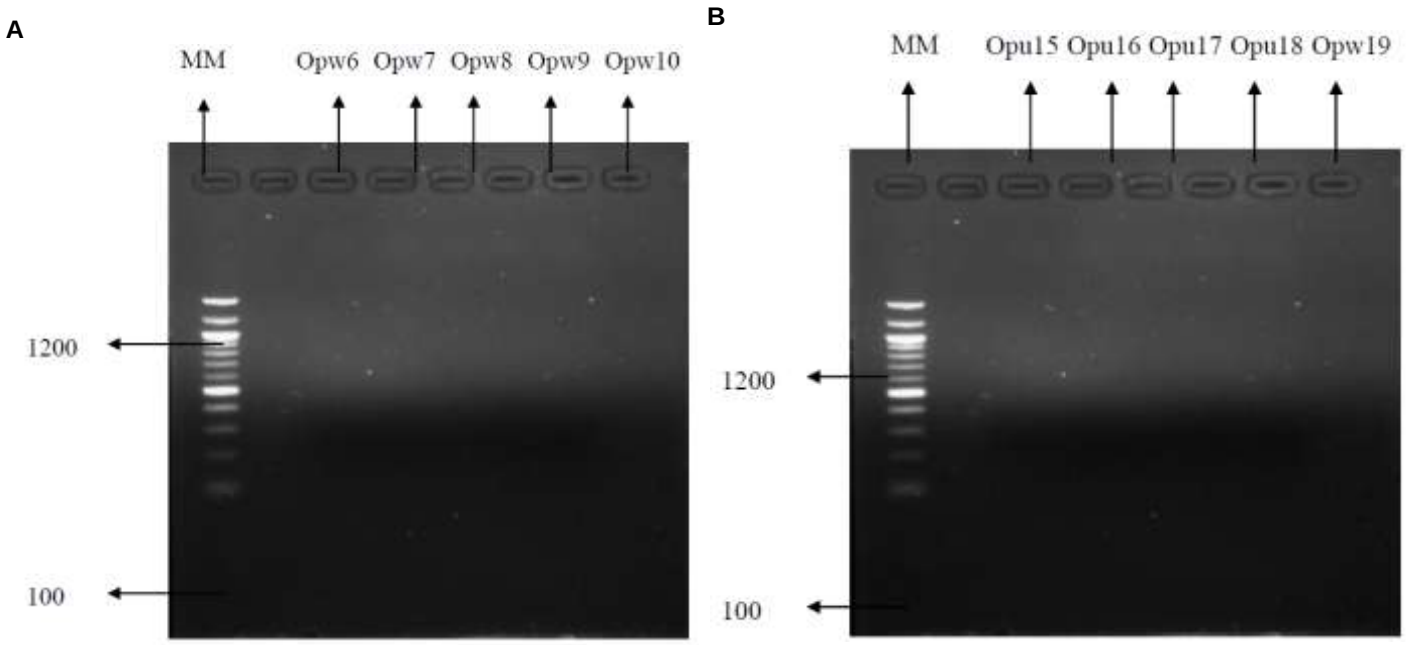

Figure 2. RAPD-PCR products of *Coleus aromaticus*.

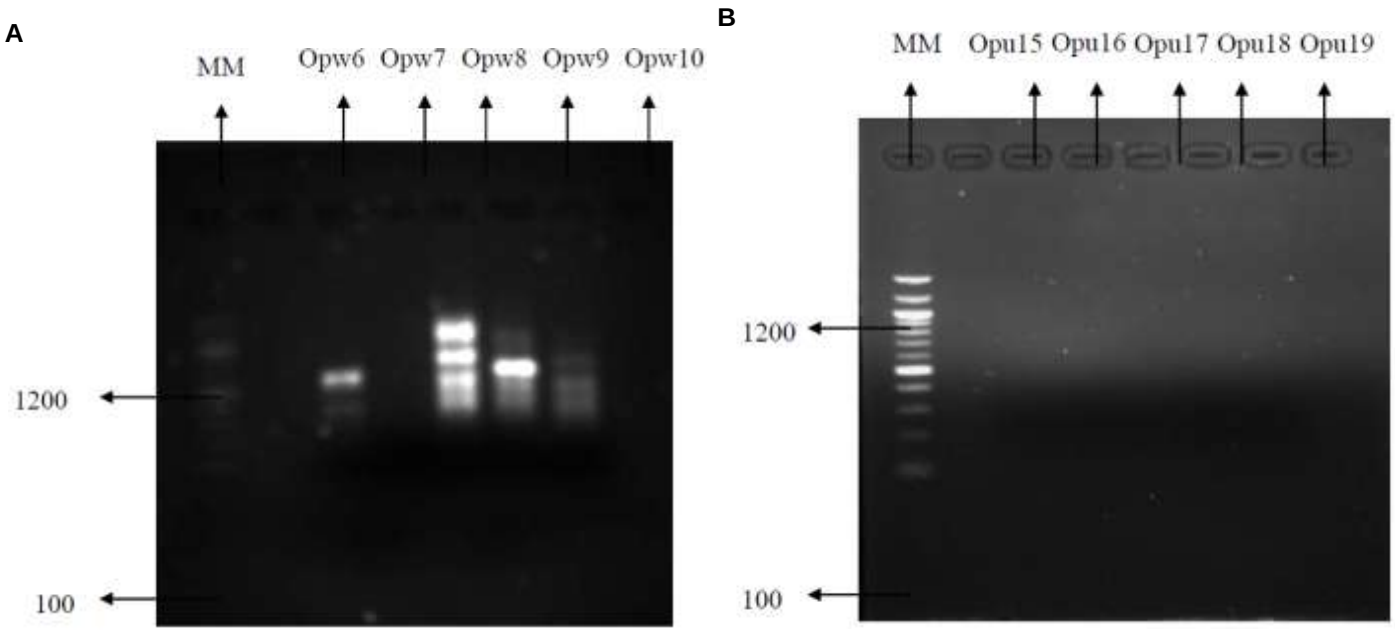

Figure 3. RAPD-PCR products of *Coleus forskohlii.*

RAPD can be considered to be essential tool in cultivar identification (DNA typing), assessment of genetic variability and relationships management of genetic resources and biodiversity, studies of phylogenetic relationships and in genome mapping (Hasibe et al., 2009). An attempt was made to analyse genetic variability among coleus species by Randomly Amplified Polymorphic DNA (RAPD) technique. DNAs of three *coleus* species were isolated in Phenol chloroform method and tested by Qualitative and Quantitative methods in order to obtain good quality and DNA for analysis.

The result provided further evidence that RAPD technique offers reliable method for characterizing variations among species, within a species and among population and is in agreement with the findings of

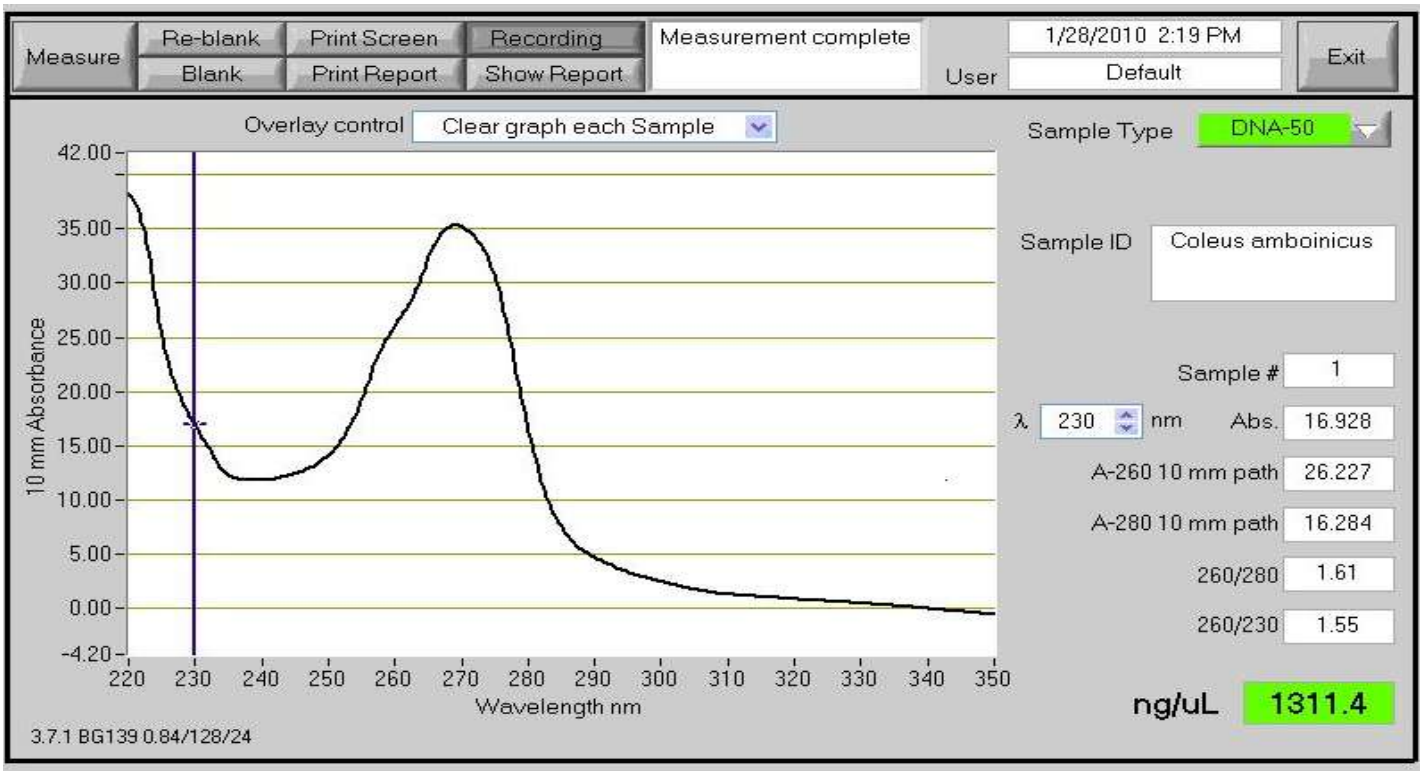

Figure 4a. Quantitative analysis of *Coleus amboinicus* DNA by Nanodrop spectrophotometry.

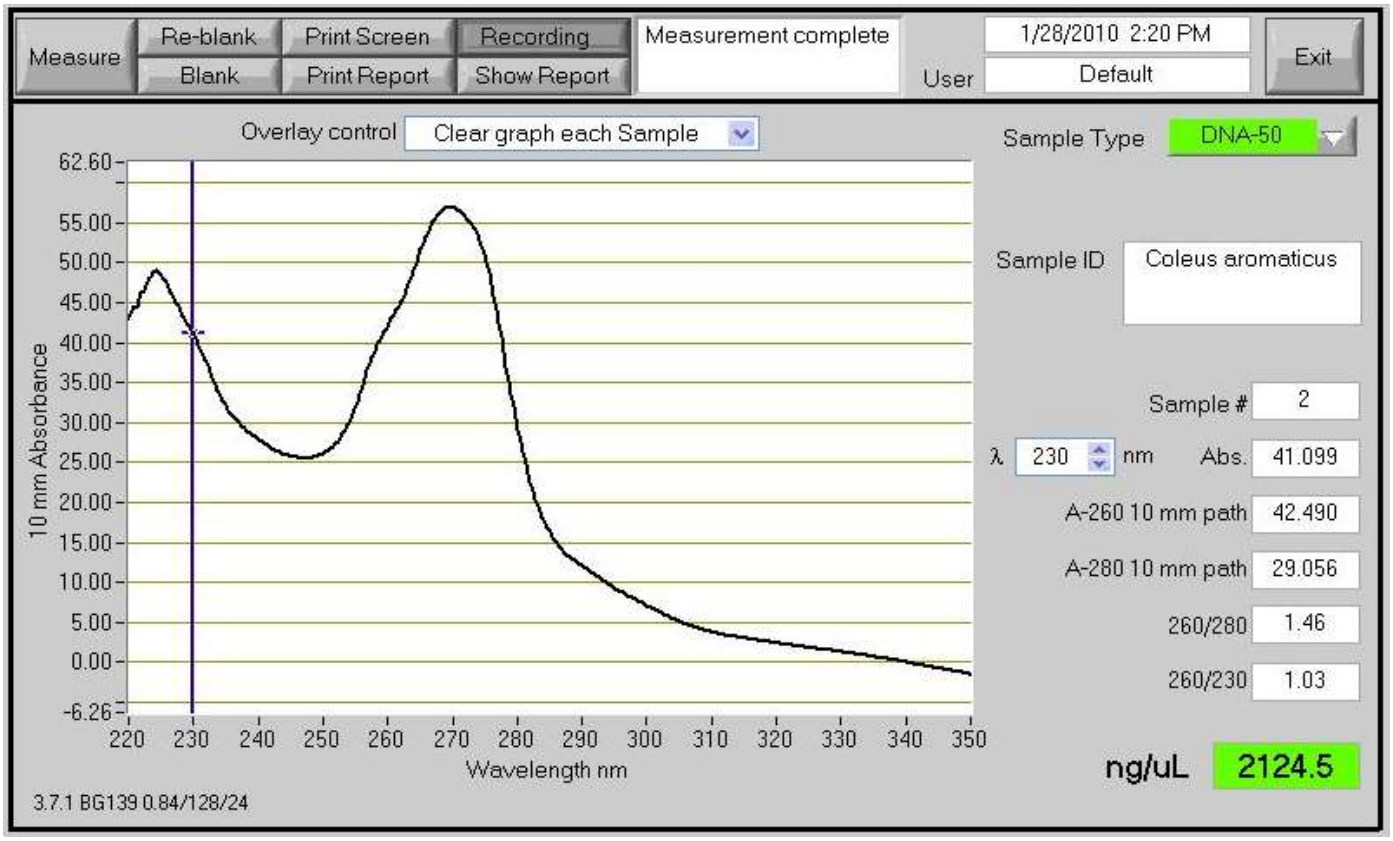

Figure 4b. Quantitative analysis of *Coleus aromaticus* DNA by Nanodrop spectrophotometry.

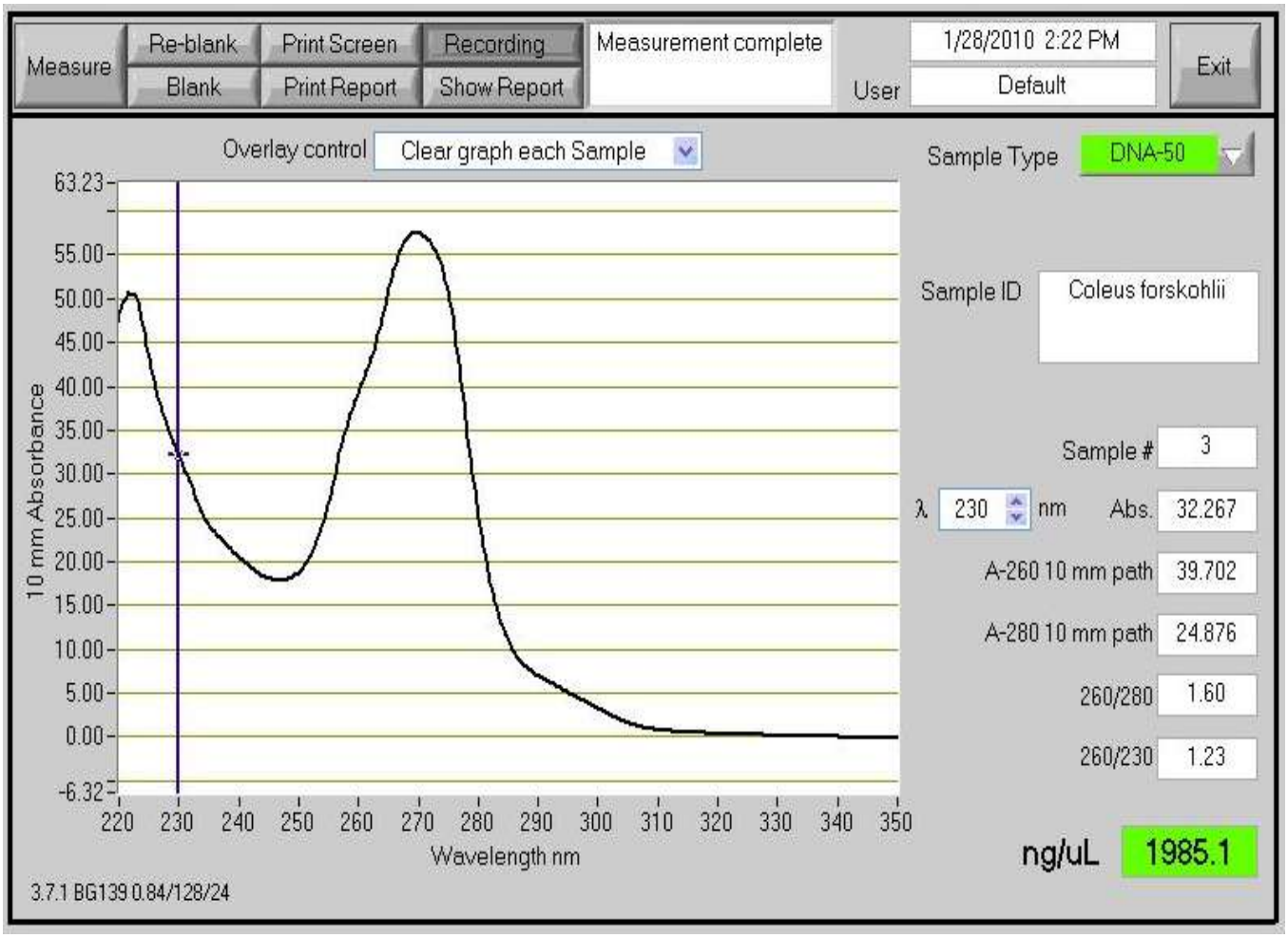

Figure 4c. Quantitative analysis of *Coleus forskohlii* DNA by Nanodrop Spectrophotometry.

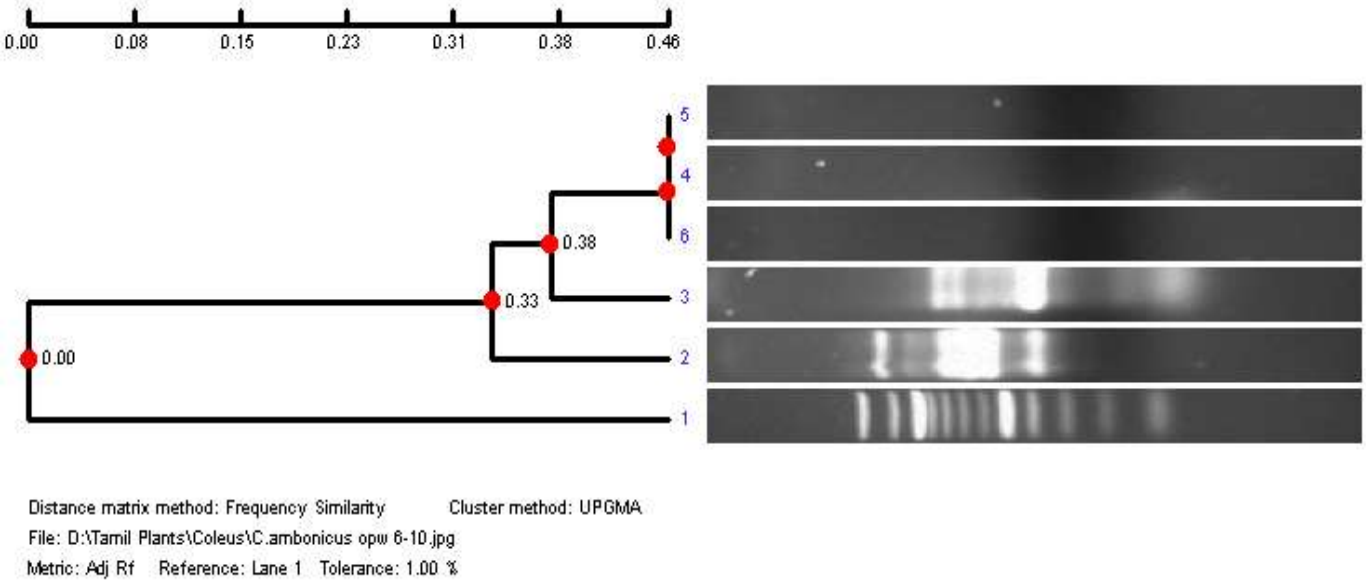

Figure 5. Unweighted pair group method with arithmetic mean (UPGMA) analysis of *Coleus* sp.

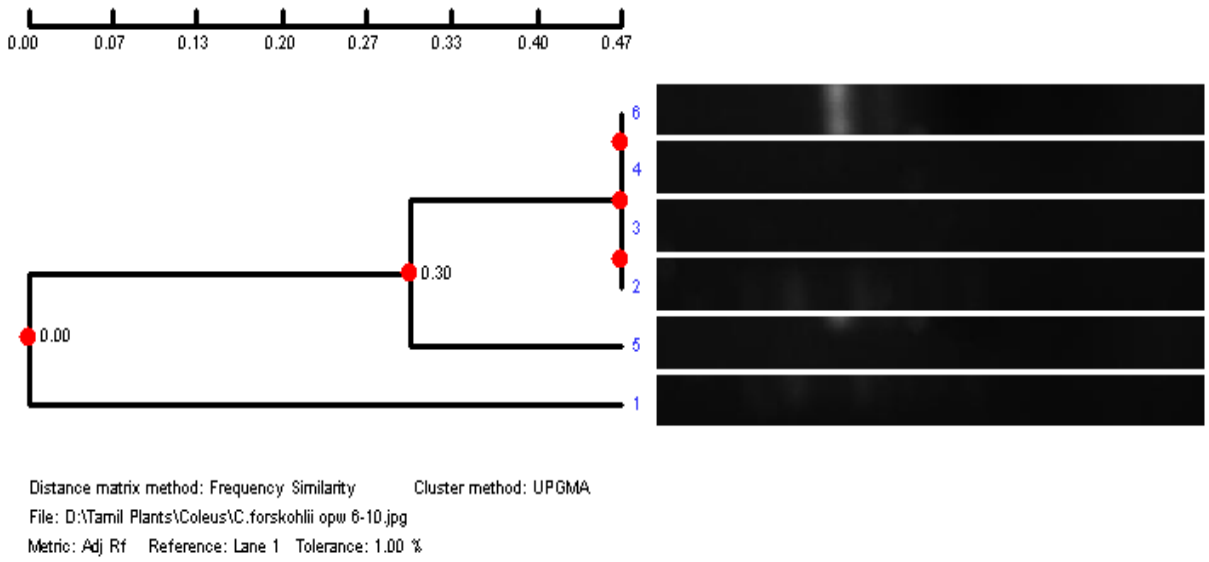

Figure 6. Unweighted pair group method with arithmetic mean (UPGMA) analysis of *Coleus* sp.

Neelambra et al. (2009).

Ten random primers were used in this study these primers generated polymorphic amplification fragments that were clear and highly reproducible but showed different intensity after illustrated under UV.

The results of RAPD showed that we need to take individuals from more different populations so as to preserve their diversity for the future. The study also confirms the suitability of RAPD as a reliable, simple, easy to handle and elegant tool in molecular diagnosis of different accessions of an important medicinal plant. Concurrently, it is also proved that the entries which were found to be similar in taxonomical classification based on morphological characters.

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