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Research Article

RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS IN *CURCULIGO ORCHIOIDES* GAERTN, AN IMPORTANT ENDANGERS SPECIES

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ABSTRACT

Curculigo orchioides Gaertn is an endangered rasayana herb which is important ingredient of many Ayurvedic preparations. The present study was formulated to access the genetic diversity within each population, among the populations and also in micro propagated plantlets of *Curculigo orchioides* Gaertn using random amplification amplified polymorphic DNA (RAPD) marker. Plants were collected from four different provenances of Tamil Nadu, India, and ten from in vitro produced plantlets. Out of the 10 RAPD primers, 6 were scored number of bands, among them Primer 1 generated 43 bands and same time primer 9 was generated 2 bands only. The band ranged from 150 to 3,000 bp in size. Number of polymorphic bands were observed when used primer 2 and 4. The monomorphic bands were highly observed when used primer 6. The number of bands in the selected primers varied from 3 to 8 bands. Genetic stability was determined based on the bands appearance and they showed identical to mother plant. RAPD profiles and dendrogram analysis based on the Jaccard's similarity coefficient revealed 100 % genetic similarity. Our results reveal very low level of genetic diversity in the species. These results also indicate that RAPD is a good molecular marker to study the genetic diversity of these species.

Keywords: *Curculigo orchioides* Gaertn, genetic diversity, RAPD, Dendrogram, Molecular marker.

INTRODUCTION

Curculigo orchioides Gaertn is an endangered rasayana herb which is popularly known as "kali Musli". The plant is native in India, and holds a special position as a potent adaptogen and aphrodisiac in Ayurvedic system of medicine (Dhar et al., 1968). It is an important ingredient of many Ayurvedic preparations and is considered to have adhrodisiac, immunostimulant, hepatoprotective, antioxidant, anticancer and antidiabetic activities (Dhar et al., 1968; Kocyan, 2007). Tissue culture can be utilized for conservation and mass propagation of selected native plants that cannot be propagated on large-scale by means of seeds and cutting to preserve endangered species.

Random amplified polymorphic DNA technology is a reliable method for characterizing variation among species, within a species and among populations (Williams et al., 1990; Gustine and Huff, 1999). RAPD profile construction has several advantages, such as rapidity of process, low cost and the use of small amounts of plant material (Jain et al., 1994; He et al., 1995; Lopez et al., 1996). In recent years, RAPD analysis has become a popular method for estimating genetic diversity in plant populations.

In the present study was find out the genetic relationship between the fields cultivated (collected from five locations in Tamilnadu) and micro propagated plants of *Curculigo orchioides* Gaertn by using RAPD technique.

MATERIALS AND METHODS

Plant Material

Micropropagated and five different locations of *Curculigo orchioides* Gaertn from Tamilnadu were collected for RAPD analysis. The plant materials were transferred to plastic bags for transport from field to laboratory. Permanent storage was at -70° C.

DNA Extraction

Ribosome were frozen in liquid nitrogen, lyophilized, and ground to a fine powder using mortar and pestle. DNA was extracted by the CTAB method according to Saghai-Marrof et al., (1984). Each DNA was then washed with chloroform : octanol (24:1), precipitated with isopropanol and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). RNA was removed by addition of 0.5 mg of RNAse A per sample. DNA concentration was estimated by comparing band intensity with known DNA standards on an agarose gel.

DNA Quantification

UV Spectrophotometric method: Nucleic acids have an absorption maximum at 260nm. Most samples contain contaminates such as proteins and single stranded DNA/RNA that absorb maximally at 280nm. The equation for calculating DNA in the presence of contaminates is A [260] /A[280] = pure dsDNA, the higher the ratio, the more pure the DNA sample. It is acceptable to have a ratio between 1.8 and 2.0.

DNA amplification

Amplification reactions of 25µL contained: 10mM Tris-HCl (pH 8), 50mM KCl, 2.5mM MgCl2, 0.1mM of each deoxynucleoside triphosphate, 0.1mM of one oligonucleotide decamer primer (Operon Technologies, CA, USA), 30 ng of genomic DNA, and one unit of Taq DNA polymerase. The reactions were conducted in a thermocycler model 9600 from Perkin-Elmer Cetus (Norwalk, Conn., USA) programmed for 40 cycles, each consisting of one denaturation step (94 °C for 15 sec), one annealing step (35 °C for 30 sec), and one extension step (72 °C for one min). After the 40th cycle one extra extension step was performed for 7 min at 72 °C. Amplification products were then separated on 1.2% agarose gels containing ethidium bromide, visualized under UV light and photographed using Polaroid film type.

Agarose gel electrophoresis: Agarose gel electrophoresis was carried out in a horizontal submarine electrophoresis unit. 30ml of 1.5% agarose gel was prepared with 1X TBE (Tris Borate Ethylene Diamine Tetra Acetic Acid) buffer (do not mix) instead the content was heated to get clear solution for casting agarose gel. After cooling the solution, 7µl of staining dye solution (Ethidium bromide) was added into the casting system.

The gel was allowed to solidify and carefully dissembled from the casting system without disturbing the wells. It was placed in 1XTBE buffer filled electrophoresis tank (the buffer level should be above gel). 5µl of sample DNA was mixed with 2µl of gel loading dye and then loaded to the gel and simultaneously loaded 3µl of DNA marker was loaded which was provided in the nearby well. The power card terminals were connected at respective positions; the gel was run at 50V, till the gel loading dye migrate more than half the length of gel. The unit was switched off and the isolated DNA visualized (amplified DNA) under UV transilluminator.

Data analysis

RAPD bands sizes were designated as amplified bands, and bands were shared as diallelic characters (present = 1and absent = 0). The number of polymorphic bands is calculated for each population. Similarity matrices were computed based on Jaccard's similarity coefficient, using the SPSS. Calculate the SI between each sample and use UPGMA algorithm to construct the dendrogram.

RESULT AND DISCUSSION

Molecular markers are used for estimation of genetic diversity/genotyping of plants. Random Amplified Polymorphic DNA (RAPD) is well established genetic tools (Williams et al., 1990; Welsh and McClelland, 1990). The relatively low cost of the technique and requirement of only nanograms of template DNA provide advantages in the use of RAPD in fish and fish products identification (Callejas and Ochando, 1998). This technique can be used to differentiate individuals or breeding stocks with a given species but also to differentiate among different species. The conditions of the analysis can be optimized to reveal only species-specific differences, since all individuals belonging to a given species have more of their genetic material in common than with individuals from other species (Elvevoll et al., 1992).

The protocols used in this study involved use different places of plant for in vitro conservation followed by micro propagation of tissue culture plant. To validate efficiency of

Table 1: Data of RAPD and primers used in the present study and the extent of polymorphism

Primer Code	Total No. of Bands	No of Max. Bands	No. of Min. Bands	No of Poly morphic Bands	Poly morphism (%)	No of Mono morphic Bands	Mono morphism (%)
Primer 1	43	12	10	2	5	7	16.2
Primer 2	37	11	8	7	19	2	5.4
Primer 3	36	10	7	4	11.1	4	11.1
Primer 4	41	11	9	7	17	3	7.3
Primer 5	28	8	6	5	18	1	3.5
Primer 6	38	10	9	2	5.2	8	21

Banding patterns of Curculigo orchioides isolates obtained using RAPD primers OPA 01, 03, 09 and OPA 10;

(Lane 1 = Cudalore; lane 2 = Micropropagated; Lane 3 = Trichy; Lane 4 = Madurai; Lane 5 = Local)

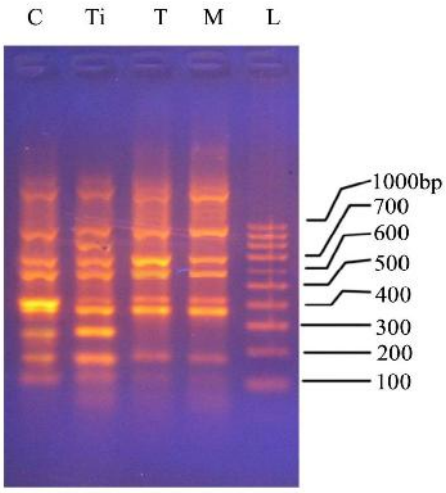


Figure 1

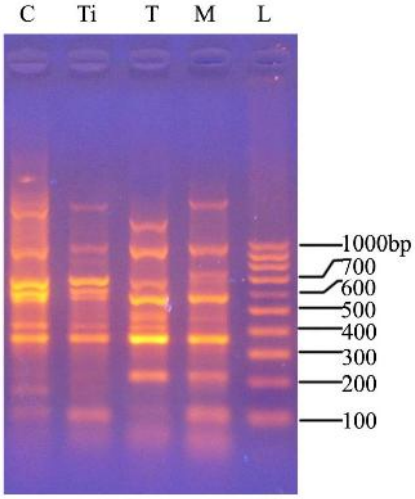
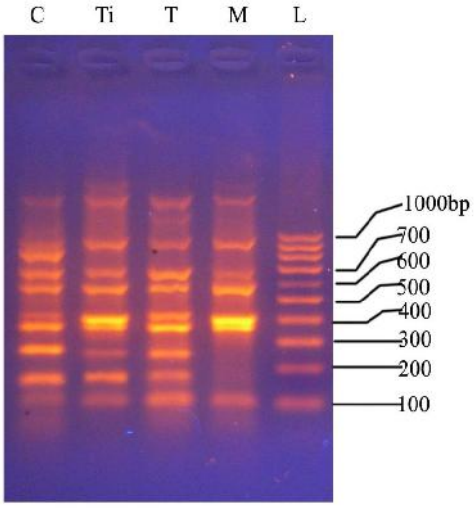


Figure 2



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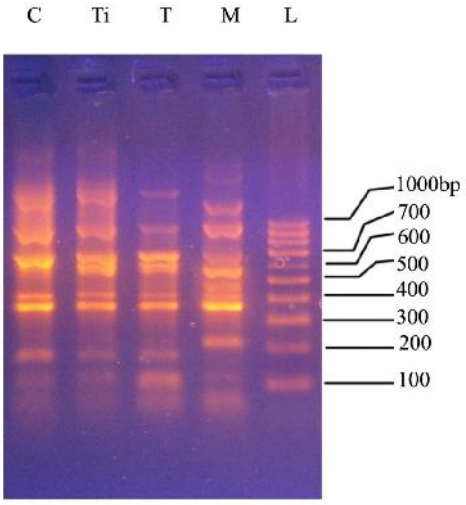


Figure 4

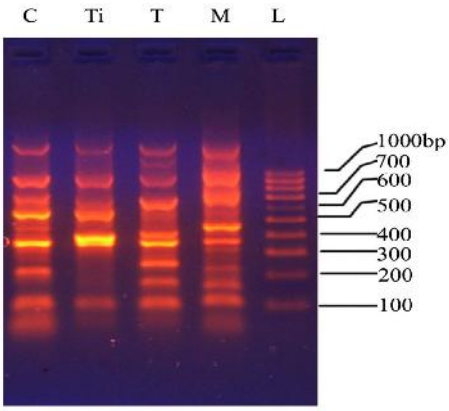


Figure 5

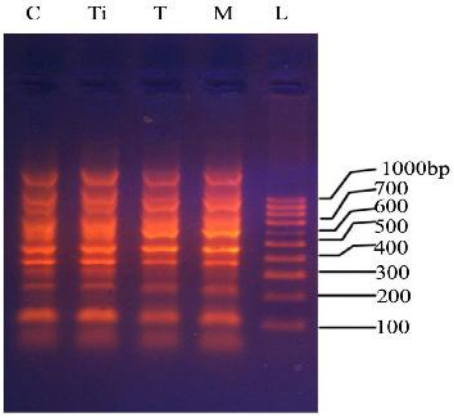


Figure 6

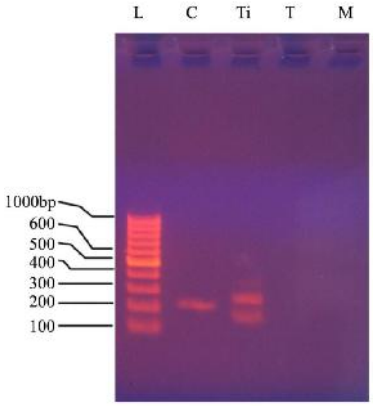


Figure 7

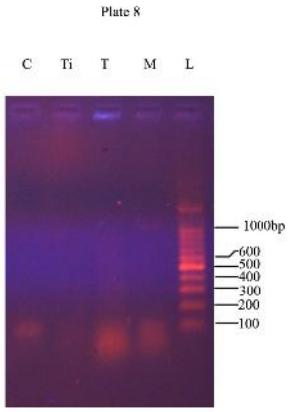


Figure 8

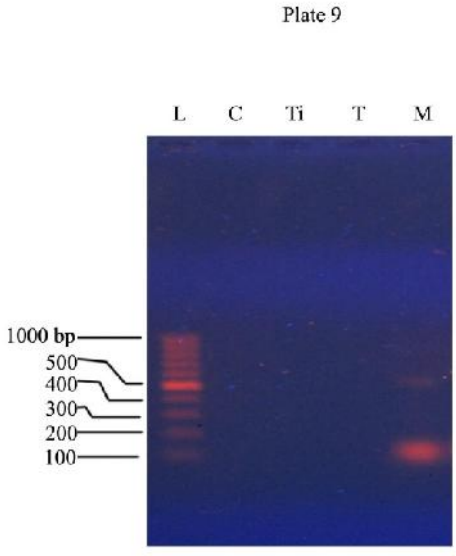


Figure 9

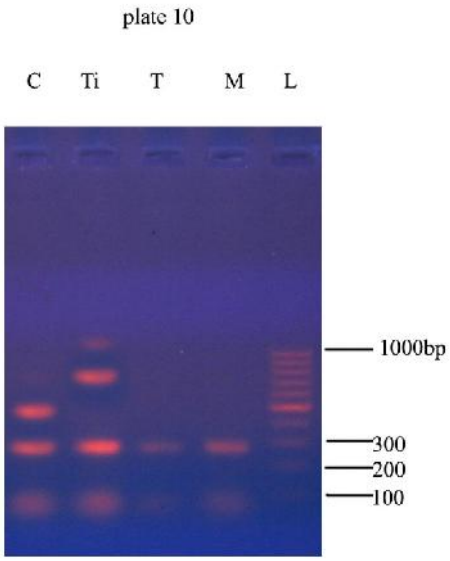
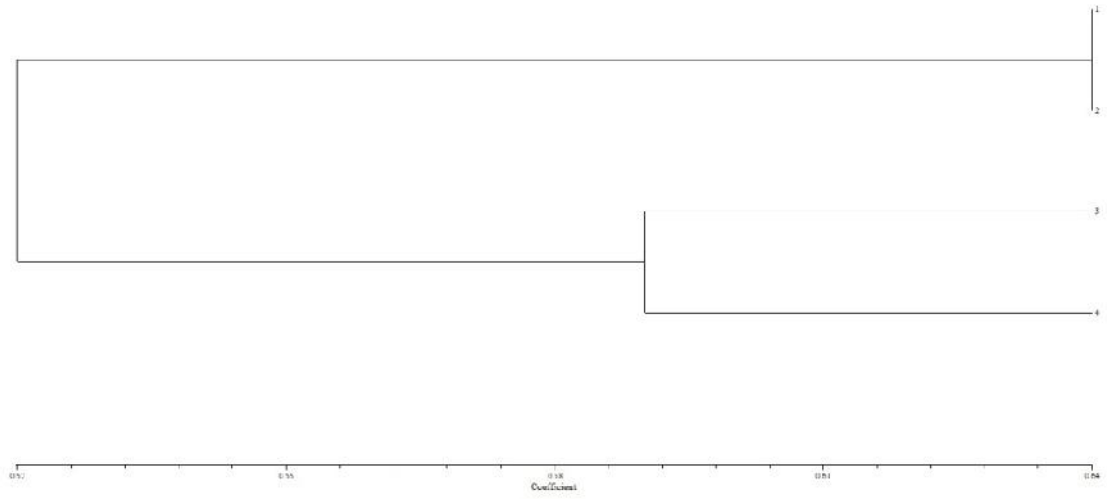


Figure 10

Dendogram based on an allelic banding pattern obtained from RAPD markers



in vitro conservation, it was essential to ascertain the genetic status of resultant progeny. RAPD markers were employed to characterize the genetic similarity of the mother plant and its derivatives. Out of the 10 RAPD primers, 6 were scored number of bands, among them Primer 1 generated 43 bands and same time primer 9 was generated 2 bands only. The band ranged from 150 to 3,000 bp in size. Number of polymorphic bands were observed when used primer 2 and 4. The monomorphic bands were highly observed when used primer 6. The number of bands in the selected primers varied from 3 to 8 bands. Genetic stability was determined based on the bands appearance and they showed identical to mother plant. RAPD profiles and dendrogram analysis based on the Jaccard's similarity coefficient revealed 100 % genetic similarity. The cluster analysis was done by UPGMA. The Dendrogram of the cluster was revealed of 4 samples. The multiple clusters of maximum similarity are formed between 1 and 2. In this study low similarity was observed between 4 and other samples.

Several reports are available to demonstrate the use of RAPD markers for determination of genetic variation in plants. Jain et al. (2003) studied molecular diversity in *Phyllanthus amarus* by RAPD profiling of 33 collections from different location using MAP primers. Mathur et al. (2008) studied genetic fidelity of micro-cloned progeny of *Chlorophytum borivilianum*. They scored 79 amplified reproducible monomorphic bands with three different sets of 24 decamer primers (14 MAP, 3 OPO, 7 OPA primers).

Khanuja et al. (1999) used 60 random primers to analyze 11 accessions from six taxa of Mentha. Nanda et al. (2004) used forty primers and selected 17 primers on the basis of their ability to detect distinct, clearly resolved and polymorphic amplified product for the analysis of six species of Acacia. They found high degree of diversity (70%) within the six tree species of Acacia. Abd-El-Haleem et al. (2009) reported genetic analysis and RAPD polymorphism in Wheat Genotypes. Naugzemys et al. (2006) reported genetic variation and relationship among 39 accessions of *Lonicera caerulea* and one accession of *L. xylosteum*. Batistini et al. (2009) evaluated the genetic diversity of seven populations of *Anemopaegma arvense*, using random amplified polymorphic DNA markers.

The present study is report that provides genetic information of *Curculigo orchioides* Gaertn in different places and microprobagated. The level of RAPD variation in different places of *Curculigo orchioides* Gaertn has been investigated. The majority of RAPD variations in these different places were between the different places rather than within different places. It is suggested that RAPD markers could be successfully applied for detecting genetic variability in natural population of *Curculigo orchioides* Gaertn. Moreover RAPD marker will have a major impact on the conservation and improvement of a *Curculigo orchioides* Gaertn.

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