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Development of random amplified polymorphic DNA markers for authentication of *Croton tiglium* Linn.

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ABSTRACT

Background: *Croton tiglium* Linn., commonly known as *Jayapala*, in Ayurvedic pharmacopoeia, is well-known for its purgative action. In the herbal raw drug market, seeds of *Baliospermum montanum* Blume., *Ricinus communis* Linn. and *Croton roxburghii* Wall. etc. are sold in the name of *Jayapla* seeds due to their morphological similarities. Hence their identification through molecular characters is need of the hour. **Aim:** Present study aims with the molecular characterization of young leaves of *C. tiglium* Linn. **Materials & Methods:** Fresh young leaves of *C. tiglium* Linn. were collected from its natural habitat Udupi, Karnnataka, during January 2017. It was used for molecular characterization and DNA fingerprints, by standard and most convenient Random Amplified Polymorphic DNA (RAPD) markers at Food testing laboratory, Junagadh Agriculture University, Gujarat, India. **Results:** All the primers gave good band patterns. Primer 5, 6, 8, 11 and 13 showed more number of light and bright bands matching characters with plant. **Conclusion:** Observed RAPD marker can be used to differentiate genuine as well as adulterated samples. The results may be used for the further research purposes and also required DNA Barcoding studies for further authentication.

Keywords: Adulteration, Croton tiglium, Dantibeeja, DNA, Jayapala, RAPD.

INTRODUCTION

Medicinal plants have been used worldwide, for centuries, to maintain health and to treat diseases, more so chronic diseases. However, adulteration and use of spurious materials as substitutes have become a major concern for users and industry for reasons of safety and efficacy. Therefore, authentication of medicinal plants is of utmost importance. Morphological[1], anatomical[2], chemical[3] and DNA markers[4] solve the problem by differentiating the genuine material from the adulterants, substitutes and spurious drugs[5]. Recently, Random Amplified Polymorphic DNA (RAPD) method has been used for the estimation of genetic diversity of medicinal plants and certain plants have been reported and recounted for their molecular characterization through RAPD markers[6,7]. *Croton tiglium* Linn., commonly known as *Jayapala*, in Ayurvedic pharmacopoeia, is well-known for its purgative action[8]. In the herbal drug market, seeds of *Baliospermum montanum* Blume., *Ricinus communis* Linn., *Croton roxburghii* Wall. and *Jatropha curcas* Linn. are sold in the name of *Jayapla* seeds due to their morphological resemblances. Hence their identification through molecular characters is needed. Review of literature reveals that the plant *Croton tiglium* Linn. has not been intended for its molecular characterisation. The present study was carried out to establish certain botanical standards for identification and authentication of *C. tiglium* through RAPD analysis.

MATERIALS AND METHODS

Collection and preservation of the sample

Fresh young leaves of *C. tiglium* Linn. were collected from its natural habitat Udupi, Karnnataka, during January 2017, identified and authenticated by local taxonomist with the help of botanical flora[9]. A sample specimen was preserved in Pharmacognosy laboratory of IPGT & RA Jamnagar (SPECIMEN NO - PHM/15-16/6204). The collected leave samples were washed under running fresh tap water to remove adherent soil and dirt.

Molecular characterization (DNA fingerprints)

Fresh leaves were used for molecular characterization and DNA fingerprints, by standard and most convenient RAPD method[10]. The RAPD reaction was performed following standard procedures at

Food testing laboratory, Junagadh Agriculture University, Gujarat, India.

DNA isolation

Young leaves were selected, cut into small pieces without cutting the veins washed with distilled water and ethanol, frozen with dry ice and crushed. To that, 2 ml of plant DNA extraction buffer was added. The samples were ground thoroughly, transferred into centrifuge tube and added 10 ml plant DNA extraction buffer. 50 µl of BME added to each tube mixed well. Incubated at 65ºC for 1 hour with intermittent mixing and centrifuged for 15 minutes at 10 K (10000 rpm). Supernatant was transferred carefully into fresh tube and added equal volume of chloroform and mixed well. Centrifuged for 15 minutes at 10 K (10000 rpm). Aqueous layer was carefully pipetted into fresh tube and precipitated with isopropanol. DNA pellet suspended in 300 µl of TE and subjected to column purification.

Column purification

Silica spin columns and buffers were from Qiagen. The column was placed in collection tube, 400µl of equilibration buffer was added to the column and centrifuged at 10000 rpm for 1min. Collected buffer was discarded. 400 µl of equilibration buffer was added to the DNA samples, mixed and loaded into the column (This step was repeated till the DNA sample was completed). Flow through was collected. 500 µl of wash buffer 1 was added, centrifuged at 10000 rpm for 1minute and buffer was collected. 500 µl of wash buffer 2 was added, centrifuged at 10000 rpm for 1 minute and buffer was collected. The empty column was centrifuged with collection tube to completely remove the wash buffer for 2 minute. 50 µl of elution buffer was added to the column placed in new collection tube. Incubated at room temperature for 2 minutes and centrifuge at 10000 rpm for 1 minute and eluted sample was saved (elution 1). Previous step was repeated (elution 2). Quantization of eluted DNA samples was done by loading into the agars gel.

RESULTS AND DISCUSSION

According to WHO general guidelines for methodologies on research and evaluation of traditional medicines, first step is assuring quality, safety and efficacy of traditional medicines for correct identification. RAPD has frequently been used for the detection of genetic variability in plants. Advantages of this method are its rapidity, simplicity and lack of need for any prior genetic information about the plant. The RAPD patterns are consistent irrespective of the plant source or age[11,12]. RAPD has been successfully utilized for the identification of medicinal plants[13,14] and herbal medicinal components[15]. In the present article *C. tiglium* Linn. was chosen for the identification through RAPD technique.

The fingerprinting patterns of *C. tiglium* Linn, seen as vertical columns with horizontal light bands on a dark background have been depicted in the Figure 1. For the analysis of DNA sample of *C. tiglium* Linn.15 primers were used (1 to 15 RAPD Primers mentioned in Table 1). Primers have been loaded from left to right. Figure 1 for *C. tiglium* Linn. contains primer 1 to 15 with DNA samples for analysis. In DNA sample of *C. tiglium* Linn. all primer give result except primer 7(OPO-04) and 12(OPP-08). The range of band size was observed from above 200 to 1500bp. All the primers showed the bright bands mostly at the range of 500bp onwards. Primers 1,2,3,5,6,8,11,13,14,15 showed very clear light bands at 500bp and less number of dark bands. Whereas the 1000bp primer 5 (OPN-04), 8 (OPO-06), 10 (OPO-10) are showed clear bright bands and light bands. At 1500bp primer 6 (OPN-09), 8 (OPO-06), 13 (OPR-05) showed the bright bands. Primer 5 (OPN-04), 6 (OPN-09), 8 (OPO-06), 11 (OPP-01) and 13 (OPR-05) showed larger number of light and bright bands matching characters with plant. Ranges between 500 to 1500bp very few bands are observed in primer 1 (OPM-02), 7 (OPO-04), 9 (OPO-07), 12 (OPP-08). These primers are very poor in nature. There are 24 bright bands exerted from the 15 primers and many light and dark bands. The bright bands exerted from the other 15 primers will support the base plant *C. tiglium*at the range of 200 to 1500bp.

Table 1: List of RAPD primers used for the analysis of *C. tiglium* Linn. DNA samples.

Sr. No.	Primer	Sequence 5'-3'
1	OPM-02	ACAACGCCTC
2	OPM-04	GGCGGTTGTC
3	OPM-05	GGGAACGTGT
4	OPN-01	CTCACGTTGG
5	OPN-04	GACCGACCCA
6	OPN-09	TGCCGGCTTG
7	OPO-04	AAGTCCGCTC
8	OPO-06	CCACGGGAAG
9	OPO-07	CAGCACTGAC
10	OPO-10	TCAGAGCGCC
11	OPP-01	GTAGCACTCC
12	OPP-08	ACATCGCCCA
13	OPR-05	GACCTAGTGG
14	OPR-06	GTCTACGGCA
15	OPR-09	TGAGCACGAG

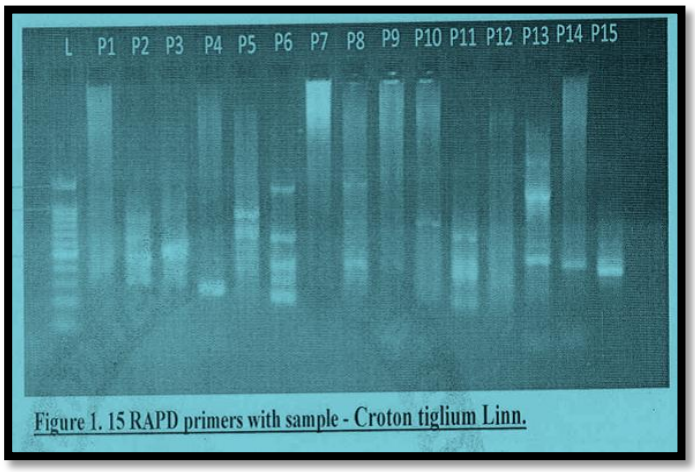

Figure 1: RAPD primers of *C. tiglium* Linn.

CONCLUSION

Establishing the standards is an integral part of establishing the correct identity and quality of a crude drug. The unique bands obtained in Polymerase Chain Reaction (PCR) amplification are clearly discriminated having many bright and light bands indicating the genuinity of the plant *C. tiglium* Linn. Observed RAPD marker can be used to differentiate genuine as well as adulterated samples. The results may be used for the further research purposes and also required DNA Barcoding studies for further authentication.

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