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RESEARCH ARTICLE

MOLECULAR MARKER STUDIES OF SELECTED MEDICINAL PLANTS FOR ASSESSMENT OF GENETIC DIVERSITY

A. RAJALAKSHMI[1], N. KRITHIGA[1] AND A. JAYACHITRA*[1]

[1]Department of Plant Bio-technology, School of Biotechnology, Madurai Kamaraj University, Madurai- 6250021, India. Email: jchitra21@gmail.com

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ABSTRACT

Objective: The aim of this study is to examine the total antioxidant activity and Genetic relationships between six different medicinal plants were analysed.

Method: The total antioxidant were analysed by using DPPH Photometric assay. The genomic DNA and RAPD Work were analyzed in selected medicinal plan using standard method. Mathwork software was used to draw the dendogram.

Result: The results observed in the present study are Out of the 5 selected plants showed high antioxidant activity followed by *Clitoria ternatea* blue leaves, *Solanum nigrum* blue Berries, *Syzygium cumini, Clitoria ternatea* white leaves, *Solanum nigrum* Red berries, *Phyllanthus emblica*. The *Syzygium cumini* has the maximum antioxidant property this was confirmed by using DPPH photometric assay Figue 1. Isolation of genomic DNA from six different selected medicinal plants by using Random Amplified Polymorphic DNA (RAPD) markers and analyse its genetic diversity. A dendrogram was constructed using Euclidean distance methods. Based on the number of bands the medicinal plants were grouped to form1-4 clusters.

Conclusion: To analyse it evolutionary process.

Keywords: Antioxidant activity, RAPD, Genetic Diversity, Medicinal plants.

INTRODUCTION

Clitoria ternatea Linn., commonly known as Shankupushpam, belongs to the family *Fabaceae* and is propagated through seeds. It is a perennial twinning herb with blue and white flowers. The parts used for medicinal purposes include roots, leaves and seeds. *Clitoria ternatea* is widely used in traditional systems of medicine as a brain tonic [1]. The blue flowered bearing plant of *Clitoria ternatea* is recommended as an herbal remedy to treat wounds, bronchitis, diarrhea, dysentery and diabetes. Its root can relieve inflamed wounds [2] . Amla which is known as *Emblica officinalis* is an Indian herb which is extensively used in ayurvedic system of medicine. Amla is a prestigious herb finds it mention in charaksamhita as a rasayan. Rasayan is a thing that prevent aging and promote longevity. Extensively used herb in making ayurvedic medicines because of its miraculous actions. According to ayurvedic doctors regular usage of Amla will make you live more than 100 years like a youth. Amla is supposed to rejuvenate all the organ systems of the body, provide strength and wellness. It keeps us away from all the diseases by boosting our immune system. It is believed by ayurvedic practitioners that if an individual regularly takes amla he can live up to an age of 100 without suffering from any type of ailments. This technique [3] has amplified the possibility of polymorphisms analysis and provides a screening method to identify region of genomic amplification, deletion or rearrangement without the need of previous knowledge of genes and or genomic sequence being investigated. It has been successfully used for typing the geographical variants in *Schistosoma mansoni* [4]. Pulverizing plant tissues under liquid nitrogen is an ideal condition for DNA extraction. Succulent tissues and leaves of some plants dipped in liquid nitrogen turn to stone-like tissues that are very difficult to crush and grind into fine powder. Avoidance of freeze grinding tissues before addition of extraction buffer was recommended, especially for tissues with high contents of water [5].

MATERIALS AND METHODS

Plant sample collection

The medicinal plants such as *Clitoria ternatea blue leaves, Solanum nigrum blue Berries,* and *Syzygium cumini*, *Solanum nigrum Red berries, Phyllanthus emblica,* were collected from local area of the south region near Nagamalai hills.

ANTIOXIDANT ACTIVITY: [6]

DPPH Photometric Assay

Principle

The ability of the leaf extracts to bleach DPPH can be quantified using a spectrophotometric assay, the extent of scavenging causing a proportionate change in the absorption at 518nm.

REAGENTS

1. DPPH (0.3 mM in methanol)
2. Methanol

Procedure

An exact amount (0.5ml) of the methanolic solution of DPPH was added with 20 µl of the leaf extracts in the different solvents and the crude aqueous extract (corresponding to 4mg) and 0.48ml of methanol, and allowed to stand at room temperature for 30 minutes. Methanol served as the blank. After 30 minutes, the absorbance was measured at 518nm and converted into percentage radical scavenging activity as follows:

$$\text{Scavenging activity (\%)} = \frac{A_{518}\,[\text{sample}] - A_{518}[\text{blank}]}{A_{518}\,[\text{blank}]} \times 100$$

DNA ISOLATION

DNA extraction and purification Genomic DNA from young leaves of species was isolated using CTAB (Cetyl trimethyl ammonium bromide) method [7] with the following modification: 3.0 g of young leaf sample was ground to a very fine powder in liquid nitrogen using a mortar and pestle. The resulting powder was dispersed in a 50 ml sterile centrifuge tube containing 25 ml of prewarmed (60°C) DNA extraction buffer (100 mM Tris HCl, pH 8.0; 20 mM EDTA, pH 8.0; 1.4 M NaCl, 2% w/v CTAB, 0.2% 2-mercaptoethanol and 2% polyvinyl pyrollidone). This tube was incubated in a water bath at 65 °C for 45 min. After the incubation, 15 ml of 24:1 choloroform:isoamyl alcohol was added, mixed well with gentle inversions and centrifuged at 20,000 rpm for 20 min at room temperature. The upper aqueous layer was separated and an equal volume of pre-chilled Iso-propanol was added and gently mixed with quick inversion for DNA precipitation. The precipitated

DNA was removed using wide bore pipette tips and transferred into 1.5 ml centrifuge tubes. The tubes were centrifuged at 10,000 rpm for 5 min. The resulting DNA pellets were washed 4 or 5 times in 70% ethanol, dried under vacuum, and dissolved in 500 µl of TE buffer (10 mM Tris HCl, 1 mM EDTA).

The RNA was removed by RNAse treatment at 37 °C for 1 h. For further purification, DNA solution was extracted once with an equal volume of phenol:choloroform:isoamyl alcohol (25:24:1; pH 8.0) followed by 2 extractions with chloroform:Isoamyl alcohol (24:1). The upper aqueous phase was precipitated by adding 2 volumes of chilled absolute alcohols, pellet and dried in vacuum, and then dissolved in TE buffer.

DNA QUANTIFICATION

UV-Visible spectrophotometer was used to measure the absorbance of isolated genomic DNA at *280/260 nm.*

Primers

Random primers were used for analysis of the amplified genomic DNA from selected medicinal plants. The single primers length is 10bp long and their sequences are 5' TTCGAGCCAG 3' used.

RAPD PCR Analysis

Principle

The genomic DNA reacts with primers. Undergoes denaturation anneling and undergoes to renaturation to give an amplified product.

Procedure

Amplification reaction was performed contains a template 1.5µl, primer 1µl enzyme master mix 12.5µl and double deionised water 10µl thus resulting in a final volume of 25µl. The amplification was performed in a DNA thermal cycler. The mixture was followed by pre-denaturation at 94˚C for 5 min, 30 cycles at 94˚C for 1min annealing 34˚C for 1 min and extension at 72˚C for 2 mins. A final extension was performed at 72˚C for 3 min [8]. The twelve microliters of the products were resolved on 1% agarose in 1X TAE buffer at 50V gels were stained with ethidium bromide photographed under ultraviolet light.

DENDOGRAM

Mathwork software were used to draw the dendogram.

RESULTS

In the present study, the leaves of five medicinal plants have been analyzed for their total antioxidant potential , DNA isolation and RAPD. The results observed in the present study are presented. Out of the 5 selected plants showed high activity followed by *Clitoria ternatea blue leaves, Solanum nigrum blue Berries, Syzygium cumini*, *Clitoria ternatea white leaves, Solanum nigrum Red berries, Phyllanthus emblica*. The *Syzygium cumini* has the maximum antioxidant property this was confirmed by using DPPH photometric assay Figure 1.

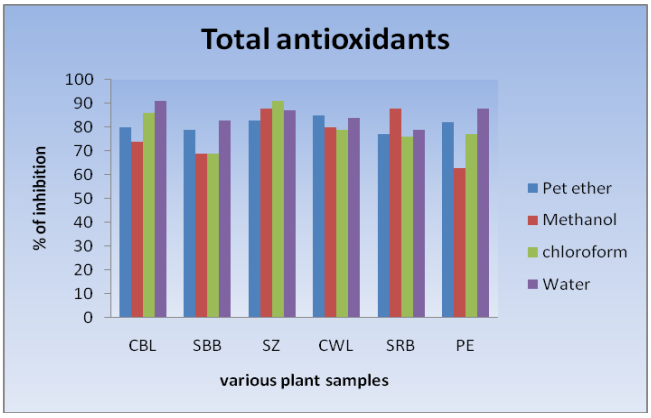

Fig. 1: DPPH Photometric Assay.

A)*Clitoria ternatea blue leaves,* ***B)*** *Solanum blue Berries* ***C)*** *Syzygium cumini*, **D)***Clitoria ternatea white leaves* **E)** *Solanum Red berries,* ***F)*** *Phyllanthus emblica,*

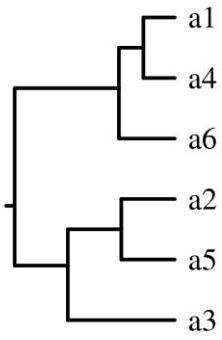

Fig. 2: Dendogram.

a1*-Clitoria ternatea blue leaves,* ***a2****- Solanum blue Berries,* ***a3*** *- Syzygium cumini*, **a4***-Clitoria ternatea white leaves* **a5**- *Solanum Red berries,* ***a6****-Phyllanthus emblica*

The DNA isolation was done by using the standard CTAB protocol. The isolated DNA was loaded in 1% gel. The gel was views. The clear distinct bands were seen. The isolated genomic DNA was purified and it was used for RAPD-PCR where a single primer sequence of decamer was choosed. It showed amplification in all the samples. This shows that single primer was able to amplify 6 different plants DNA. The RAPD gel was viewed in UV-Transilluminator by observing the gel the bands in the gel the dendogram was plotted by using Matrix (Mathwork Software). The six different plants are used to draw the dendogram based on the matrix scoring of the band in the gel using UGMA matrix system.

DISCUSSION

Various types of plant leaves and a number of different protocols for the isolation of DNA were tested in order to obtain good quality DNA for PCR reactions. When fresh or frozen leaves of plants collected and used for the isolation of DNA, no positive result in PCR reaction was obtained regardless of the isolation protocol being used. This was probably due to the accumulation of large amounts of secondary metabolites in old plant material, as previously reported [9].RAPD method is less expensive and easy to perform but reproductibility depends on choosing the right primers, whereas RFLP is reproductive but require a large amount of DNA[10]. A positive PCR reaction was also obtained when DNA was isolated from leaves taken from the dried at 4°C. It was found that the age and quality of plant material were of major importance for a successful PCR amplification. The results of PCR reactions with different types of plant material used and different isolation protocols are used. Molecular markers can be considered to be essential tools in cultivar identification (DNA typing), assessment of genetic variability and relationships, studies of phylo-genetic relationships. The effectiveness of this depends to a large extent on the genetic information available on the germplasm under study. Because of a limited number of samples from individual populations and a small number of polymorphic RAPD profiles generated after electrophoresis of PCR products, the results of statistical analysis showed that samples belonging to the same populations, as well as outgroups on the dend-rogram, did not usually cluster together. There are many plant species in the world with medicinal properties. However, almost all MAPs are neglected or under-utilized and insufficiently documented. Cultivation of MAPs is limited and most are wildly crafted, causing degradation of natural habitats. Therefore, a need for conservation is arising for many species. Individual RAPD patterns were compared within and between sampling siter. Only reproducible well marked amplified fragments

were scored for each genotype, the present and absene of fragments were recorded as are respectively. The pairwise comparison of banding patterns was evaluated by Nei's genetic distance.

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