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Genomic DNA isolation and electrophoresis analysis of extracted compound of *Phyllanthus amarus* occurring in local area of Patna,Bihar,India

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Abstract: *Phyllanthus amarus* is an erect, annual plant growing in tropical and subtropical region of India. Plant have many medicinal properties and uses for treatment of many diseases. The identification of plant species based on the morphological characters but the genetic blue print give us the exact information for the identification of the plant species. Medicinal plants needs the accurate identification among the species belonging to the same genus.

Key Words: *Phyllanthus amarus*, Euphorbiaceae, Genomic DNA, PCR, medicinal plant.

I. INTRODUCTION

Phyllanthus amarus is a wild plant of the family Euphorbiaceae (Phyllantheceae) distinguishing characters of phyllantheceae include non-woody habit, mono or bipinnate leaves which way be simple and always without stipules, zygomorphic flowers, which is small may subregular arranged usually in racemose, free sepals and petals.The plant is distributed throughout India mainly tropical and subtropical parts of the country. Patna district is the located in Bihar and its belong to warm and temperate climate.*Phyllanthus amarus* plant commonly grow on the river bank side,roadside,railway track side etc.*Phyllanthus amarus*is an erect,annual plant growing from 10-50cm tall,but usually less than 30cm.*Phyllanthus amarus* plant is found as a weed in villages,gardens and cultivated fields.*Phyllanthus amarus* is a important medicinal plant and medicinal plant have many have many bioactive constituents i.e. alkaloids, tannins, flavonoids and phenolic compounds[1].

DNA studies recently has emerged as a simple method of validating herbal samples and differentiating species. This method allows for validation and comparison between the complex phytochemical products of herbal extract and plant-based medicine against a known or "standard" extract. In the context of herbal medicines, it allows comparison on the chemical level between herbal batches supposed to contain a single plant species[2].Due to the limitations of taxonomic and chromatographic methods of identification, biodiversity research has been trending towards DNA barcoding. DNA barcoding uses short DNA sequences between 400 and 800 base pairs as "barcoding regions" that can be used to characterize and identify species, utilizing the fact that there is a degree of genetic variation between different species within the same genus[3]. Barcoding regions must exhibit sufficient genetic divergence between species in order to be used as effective markers[4].By cataloguing the barcoding regions of as many species as possible, an organization called the Consortium for the Barcode of Life aims to create a virtual database of species, identifiers, that can be publicly accessed to match.

II. MATERIALS AND METHODS:

Patna is completely lies in the sub tropical region region of temperate zone and its climate type is humid subtropical. This type of climate is suitable for the *Phyllanthus amarus* plant growth.*Phyllanthus amarus* plant was selected for the present study and extensive field trips were carried out to collect the plants from three different distinct locations.With the help of the book, Indian Medicinal Plants[5], the identification was confirmed.

Fig:Habitat of the plant

Fig:Uppar parts of the plant

Fresh plant parts were physically extracted and then lysed with the TissueLyser System. Lysed plant cells were subjected to DNA extraction via the DNeasy Plant Mini Kit. All samples yield concentrated DNA at around 100 ng/µl concentrations following extraction.

Isolation of genomic DNA: For DNA isolation fresh and young leaf samples were collected. The collection of leaves from the three study sites. Selected 2 grams of leaves were cut into small bits and transferred to a prechilled mortar.Then after in liquid nitrogen leaf tissues was frozen and ground in to a fine powder. The powder was transferred to a centrifuge tube.Then the powder added with 10 ml of preheated (65°C) extraction buffer containing 1.5% (w/v) cetyltrimethylammonium bromide (CTAB)[6]. 10 mMTris HCL (pH & 8.0), 1.4 M sodium chloride, 20 mM EDTA and 1% v/v 2-mercaptoethannol mixed together. At the 65°C the mixture was incubated in a waterbath for 30 min with occasional mixing. Equal volume of chloroform: isoamyl alcohol (24:1) v/v was added, gently mixed for 15 min and centrifuged at 10,000 rpm for 20 min at room temperature (37°C).In the new tube clear aqueous phase was transferred.Then after equal volume of isopropanol in cold ice was added and mixed gently by inversion until the DNA was precipitated out (10-20 sec). By using a sterile bent Pasteur pipette and air dried, the precipitated DNA was hooked out. The dried pellet was dissolved in 200-500 µl of TE (10 mMTris-pH 8.0, ImM EDTA pH 8.0). The contaminant RNA was eliminated from DNA by treating the DNA sample with RNase to a final concentration of 20µg/ml.In the room temperature the sample was kept for 15 minutes.

DNA Purification: Genomic DNA, was purified by phenol: chloroform: isoamyl alcohol (25:24:1): extraction mixture. An equal volume of phenol: chloroform: isoamyl alcohol mixture was added to the DNA sample and mixed by repeated inversions.

At room temperature the mixture was centrifuged at 10000 rpm for 10 minutes and in the fresh tube aqueous phase was transferred.Then after centrifuged at 10000 rpm for 2minutes with added chloroform and the aqueous phase was transferred to another tube. To the aqueous phase 1/10thvolume of 3 M sodium acetate (pH 8.0 for genomic and plastid DNA pH7.0 for DNA fragments) and two volumes of absolute ethanol was added and pelleted by centrifugation at 10,000 rpm for 5 min. After discarding the supernatant the resulting pellet was dissolved in nuclease free water and stored at -20° C.

DNA Quantification: By the using of spectrophotometer Genomic DNA and amplified products was quantified.The diluted DNA samples (1:250) were read at 260 nm and distilled water was taken as blank. The following formula was calcuted for the amount of DNA.

Amount of DNA (µg/µl) = $\frac{OD(260)nmx\ 50xDilutionfactor}{1000}$

PCR amplification: In PCR tubes about 50ng of DNA samples were taken.It was mixed with 200iM of each dNTPs, 0.5M RAPD primer, 25 mM $MgCl_2$ 1 unit of *Taq* polymerase and reaction buffer.Ovarall the total reaction volume was made up to 25µl by nuclease free water. Using the following cycling conditions, reaction tubes were placed in MJ thermal cycler.

Initial denaturation -95^{o}C for 3 min, Denaturation -94^{o}C for 1 min,Primer annealing -37^{o}C for 1 min,Extension -72^{o}C for 1 min and 20 sec. for 40 cycles,Final extension -72^{o}C for 1 and then hold on at 4^{o}C.

Electrophoresis of samples: In this process the samples were added with dye containing TBE buffer, glycerol and bromophenol blue after the completion of PCR. Agarose gel (1.5%) was casted with TBE buffer and the samples were loaded in the wells.At the 60 volts for 4 hours electrophoresis was carried out.The gels were documented in Gel documentation system after the electrophoresis.

DNA extractions were performed on 20 mg dry samples using the DNeasy Plant Mini Kit (Qiagen). Prior to extraction the samples were lysed using the TissueLyser System (Qiagen) with glass beads. Extraction proceeded following the manufacturers protocol. The preparation of the PCR mixture was done over ice. The quantity of each component depends on how many PCR reactions are setting up. Then a 20µl reaction mixture was prepared. The final concentration of each component should be: Master-Mix - 3.0µl,10mM dNTP's -0.8µl, 100 µM Forward Primer -

0.1µl,100 µM Reverse Primer-0.1µl,Bioline Biolaselaq (5U/µl)-0.2µl,DNA-1.0µl,H_2O-14.8µl.

Assembled all components and then after, gently vortex the sample and briefly centrifuge to collect all drops from walls of tube and in the thermocycler mixture will placed,which will start the amplification process at 95°C for 3 min, then proceed at 94^0c for 30sec, 55^0c for 1min and finally 72°C for 1 min. This cycle will be repeated 5 times. Then the reaction will go back up to 94°C for 30sec, 54°C for 30 min, and finally 72°C for 1 min with a final extension of 10 minutes at 72°C. The reaction will hold at 10°C until we are ready to take out the PCR product.

Sequencing: The PCR products were sequenced by GENEWIZ Inc. Both forward and reverse sequences were sequenced to ensure accuracy. Raw sequences were cleaned with Codon Code to trim ends and locate inaccurate sequence peaks.

Sequence Analysis : Our sequencing data was analyzed through two methods: (1) neighbor-joining tree method and (2) BLAST distance method.

Trees were created with SeaView through a neighbor-joining method.[7] We pulled 61 additional matK *Phyllanthus amarus* sequences and 36 additional rbcL sequences from the National Center for Biotechnology Information (NCBI) database. These sequences were uploaded to Sea View to reconstruct our phylogenetic tree. Default settings were followed with 1000 bootstrap replicates.The distance method was done through performing BLAST nucleotide searches through NBCL. We BLASTed all cleaned sequences and analyzed the top hits. We took into consideration the max identity percentage and max score of each hit, and subsequently classified our samples based on these criteria. All specimens of *Phyllanthus amarus*used in the biochemical analysis and extractions were compared to this standard.

III. RESULTS

For studied of Genomic DNA, Genomic DNA was extracted from root, petal, bulb (stem) and leaf of *Phyllanthus amarus* with a view to loop into whether persistent induces any kind of genomic DNA imbalance presents the result of electrophoresis of genomic DNA from root and leaf respectively protocol of genomic DNA extraction. It present the electrophoresis results of restrict digested genomic DNA of *Phyllanthus amarus*. The run of 1kb ladder A – 2, Eco R_1 digest of Petal DNA Bam H_1 digest of Petal DNA.

The observation shows 1kb ladder while B-2 presents fragile root genomic DNA. Eco R_1 digests petal DNA shows two bands while Bam H_1 digests of petal DNA shows two bands. Treatment of root DNA by restriction enzymes resulted into non distinguishable bands and apparently continuous flow of DNA. Genomic DNA untreated with any band separation except the main group and all the four lanes had mass to genomic DNA separated to the same level. It proves that there has been no difference in total DNA contents of the plant by apparent increase in few chromosomes no. due to endo-duplication.

Genomic DNA separation involves extraction of DNA from hundred of the cells having same amount of DNA approximately and that is why, there would be no discernible difference in the amount of separated DNA through gel electrophoresis. To investigate into minor genomic DNA difference from cell to cell, other advance technique would be required difference from cell to cell, other advance technique would be required.In the present study, simple electrophoresis separation of genomic DNA has been observed. Bam H_1 could destabilize DNA due to additional chromosome having DNA length that had restriction site. Additional cuts lead to destabilization and free flow of root DNA. Such free flow could not be observed in case of DNA isolated from root and leaf.

IV. CONCLUSION

The identification of plant species based on the morphological characters but the genetic blue print give us the exact information for the identification of the plant species. Medicinal plants needs the accurate identification among the species belonging to the same genus. Genomic DNA study of is play important role in the identification of medicinal plant.*Phyllanthus amarus* is also an important medicinal plant and uses for treatment of many diseases. So Genomic DNA study is helpful for medicinal study.

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