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Study of the Genetic Variations in Different Variants of *Tribulus terrestris* L. in Rayalaseema Region in Andhra Pradesh

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Abstract

Tribulus terrestris (Family - *Zygophyllaceae*) is a finely known medicinal herb, usually called as palleru in Andhra Pradesh. This is seen all over the world along with India and is used in diverse systems of medicine such as diuresis, chronic cystis, calculous affection, incontinence of urine, impotency, gonorrhoea, gleet, spermetorrhoea, and phosphateurea. *Tribulus terrestris* is one of the components of *Dashamoola*, is an ancient Ayurvedic formulation made up of ten medicinal herbs which is used in various Ayurvedic medicines and as well as in chyavanprash. And used as tonic in Unani medicine. In the present study 8 different regions of *Tribulus terrestris* plant leaves were collected which is further subjected to molecular characterization by RAPD analysis. In present days RAPD is used for trace the phylogeny in both animal and plant diverse. RAPD analysis found that various mild variations with respect to different regions of Kurnool district, Rayalaseema region of Andhra Pradesh. It was observed that all 15 primers were found to generate clear and polymorphic bands. Total number of bands produced was 223 in which 159 shows polymorphic bands. The percentage of polymorphism obtained by 15 primers was 71.3%.

Keywords *Tribulus terrestris*, Genetic diversity, RAPD analysis, medicinal herb.

Introduction

Tribulus terrestris a medicinal herb. These herbs are finding and are uses in customary medicine from primitive era. Medicinal plants have an ability to produce a hundreds of chemical compounds which shows the shielding mechanism against microorganism such as bacterial, fungal diseases along with insects and plant feeders. Many phytochemical molecules with potential biological active compounds have been identified in medicinal plants. However the entire single plant contains various phytochemical compounds and also a whole plant is used as medicine. Due to the presence of derived compounds and pharmacological actions they shows medicinal potential remain unassessed by exact scientific research to define the value and safety. In plants diversity centres, India ranks 4th in Asia and 10th in the world. In 17 mega biodiversity centres of world, india is one which have an incredible wealth in a form of medicinal herbs and plants. And is a home for all 8000 medicinal plants. An increasing demand trends in food, traditional medicinal plant based medicines in pharmaceutical and cosmetic industries is utilization of natural plant extracts have been more well-liked in both developing and developed countries. Due to constantly increasing demands of herbal drugs is causing thrashing of valuable biodiversity and also build lack of raw material (1). Therefore Since there has been few studies on genetic diversity on Indian medicinal herbs (2,3) for efficient maintenance and organization of genetic resources, it is necessary to analyze the genetic composition of species in different phytogeographical regions. So for this reason, we have chosen *Tribulus terrestris*. It is normally identified as land caltrops and belongs to the family Zygophllaceae, which a tap rooted, biennial herb with diffused stem which grows in temperate climates. As it as taproot system a fine networks of rootlets can seen which are used to absorb humidity from soil and can exist in dry environment. It can also grow in any type of soil even in dry, loose, sandy mud, deserts, along a roadsides. Gokshura one of the components of Dashamoola which is widely used in Ayurveda (4,5). It is an important medicinal weed found widely distributed around the world along with India, Srilanka and West Tibet. To this plant there are various familiar names according to the area including bhakhra, puncture vine, and tack weed, palleru (Telugu), Brihat Gokhur (Sanskrit), Nerunji Mull (Tamil), Baijili (chines), khar-e-khasak (Urdu). It is also named as trikanta since the fruit has sharp thorns along its surface which is hard enough to even puncture a cycle tyre. It is used as traditional Unani and Chinese medicine for curing different health issues. Michirkand is a essential ingredient that is useful for treating urinary disorders, PCOS, prostate gland problems, kidney problems, jaundice, alopecia and heart ailments. It is also majorly helps in building muscle mass, improving brain activity and boosting the libido in both men and women. Due to the presence of highly active ingredients it is often used in folk medicine and also supplements as food and also highlights the importance of evaluating its phytopharmacological properties. As it contain saponins, steroid, flavonoids, alkaloids, fatty acids, tannins, resins carbohydrates, glycosides and proteins (6). As *Tribulus terrestris* is a rich source of steroidal saponins, of diosgenin, dioscin protodioscin it is use to treat sexual dysfunction issued in humans (7). In present days, entire plant or roots or fruits are used in large portion as tonic, dashamoolarsht and in treatment of immunostimulants, low water retention capacity, hair fall, stress, neural problems. The complete plant is useful to weight loss, glucose intolerance and in polycystic ovarian (8,9). The roots are useful in cough, asthma, internal inflammation, anemia, headache menstruation, piles, bed wetting, and eye problems. . Due to having multiple medicinal value and world-wide allocation there has been not report on the extent of genetic diversity in different locations of Kurnool district, Rayalaseema region of A.P, India. So that here we conducted the study to analysis the genetic diversity by using RAPD analysis method. It provides soaring resolution amount can be carried out on small amount of DNA (10,11). DNA markers were mostly used for genetic diversity, evolutionary, Population structure assessment, for inferring interrelationship and gene linkage mapping.

Material and Methods

Plants material

T.terrestris, sample leaves were collected form naturally developed inhabitants of eight dissimilar locations of Kurnool district and their identity was confirmed by Dr. Madhava Chetty plant taxonomist and voucher specimens were deposit in the herbarium, Department of Botany, Sri Venkateswara University, Tirupati-517202 ,India.

DNA extraction

Collect apical young, healthy and fresh leaves from the selected plant were used for the isolation of DNA by DNeasy plant mini kit method. Place the tissue (100 mg) into 2 ml microcentrifuge and add liquid nitrogen which freezes the leaves. After 30 sec liquid nitrogen evaporates to that add 400 µl of Buffer AP1. Keep the tubes in homogenizer adapter then set and fix into the clamps of homogenizer. Samples were grind for 1 min at 30HZ. Disassemble and reserve the position of the tubes. Grind again for 1 min at 30 Hz. Incubate mixtures for 10 min at 65°C then mix it for 2-3 times during incubation by inverting tube. Add 130 µl of Buffer P3 to the lysate, mix and incubate for 10 min on ice. Centrifuge for 6 min at 14000 rpm. Transfer the flow-through to a fresh microcentrifuge tube, without any disturbing the pellet. Add 1.5 volumes of Buffer AW1 along with ethanol. Mix by pipetting. Apply 650 µl of the mixture including any precipitate to the spin column sitting in a 2 ml collection tube. Centrifuge for 1 min >6000 rpm. Discard flow-through. Add 500 µl Buffer AW2 to the spin column and centrifuge for 1 min at 6000 rpm. Discard flow through. Add 500 µl 100% Ethanol to the spin column and centrifuge for 2 min or until membrane is dry on max speed. Carefully remove the spin column from the collection tube and transfer to a 1.5 or 2 ml microcentrifuge tube and pipet 100 µl of Buffer AE directly onto the membrane. Incubate for 5 min at room temperature and then centrifuge >6000 rpm for 1 minute, centrifuge the lysate for 5 min at 14000 rpm. Store at -20/80ºC (long-term) of 4ºC (short –term). Yield and purity are determined using a spectrophotometer and on an agarose gel to confirm quality and relative concentration.

RAPD analyses

RAPD assay was carried out in 25 µl reaction mixture composed of 50 ng of total cellular DNA (2.5 µl), 10nM or primer (1.5 µl), double distill water 13.5 µl,1.5 µl of Taq DNA polymerase reaction, buffer 1.2 µl and 2.5 mM of each dNTPs (DNA polymerization mix). Amplification is done in thermo cycler. The RAPD profile consisted 95 ºC for 3 mins followed by 35 cycles consisting of 94 ºC for 1 min, at 37 ºC for 1 min and 72 ºC for 1 min. A final incubation step for 10 mins also at 72 ºC was performed. Then the amplification product analysis on 0.8 % agarose gel in 10XTBE. Gels were run for 30 min-1 h at 70 V and stained with ethidium bromide. This strongly binds to DNA by intercalating between the bases. After DNA was run up to 80% of the gel distance, locations of the bands was visualized by examining the gel under UV trans-illuminator.

Results and Discussion

RAPD technique is used for detecting genetic variation and relatedness among the different genotypes, so far RAPD markers and studies were reportedly used to distinguish the genetic variation. In this technique can study at the species individual level which is closely related. Medicinal herbs were authentic by using RAPD. Importances of RAPD analysis incorporate their simplicity, rapidity and low amount of genomic DNA required. Molecular markers produce by RAPD, target different regions of the DNA in a random way. It was observed that all 15 primers were found to generate clear and polymorphic bands. Total numbers of bands generated were 223 in which 159 shows polymorphism in bands. The percentage of polymorphism obtained by 15 primers was 71.3. The number of RAPD bands was in rage of 10-17 per primer, OPC3 producing the minimum number of 6 bands where as OPC5 producing the maximum number of bands 35. On average 14.8 bands were obtained per primer and the number of amplified bands per primer was 10.6. Similarity index value derived from the polymorphic data range from 28% (TT_1 -TT_7) to 99% (TT_4 –TT_6). The dendrogram showed a closer relationship among genotypes TT_1 and TT_7 (28%) followed by TT_5 and TT_7 (32%), TT_1 and TT_3 (40%) followed by TT_5 and TT_8 (48%). The dendrogram results showed that sample 4 and 6 is distinct; this is an indication that it has traits different from others.

Conclusion

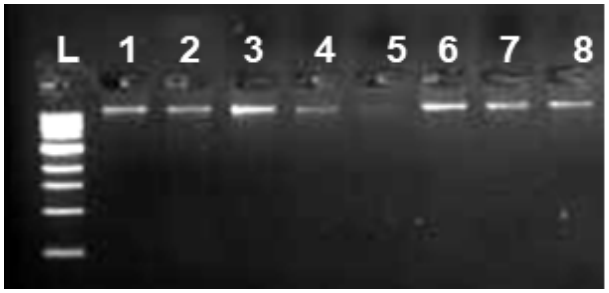
In RAPD analysis, the samples which are collected from eight different locations gives clearly polymorphic bands with each and Primers and number of bands generated through PCR amplification and polymorphism (%)

Primer Code	Primer Sequence (5'-3')	Total No of bands scored	No of amplified bands	Polymorphism (%)
Primer-OPA 1	CAGGCCCTTC	10	4	40.0
Primer-OPA 3	AGTCAGCCAC	18	15	83.3
Primer-OPA 5	AGGGGTCTTG	14	9	64.2
Primer-OPA7	GAAACGGGTG	21	15	71.4
Primer-OPA9	GGGTAACGCC	22	16	72.7
Primer-OPB4	GGACTGGAGT	9	4	44.4
Primer-OPB6	TGCTCTGCCC	7	4	57.1
Primer-OPC2	GTGAGGCGTC	15	10	66.6
Primer-OPC3	GGGGGTCTTT	6	3	50.0
Primer-OPC5	GATGACCGCC	35	29	82.8
Primer-OPC11	AAAGCTGCGG	11	9	81.8
Primer-OPC12	TGTCATCCCC	10	7	70.0
Primer-OPD2	GGACCCAACC	16	13	81.2
Primer-OPD5	TGAGCGGACA	12	9	75.0
Primer-OPD7	TTGGCACGGG	17	12	70.5
Total No. of bands		223	159	71.3
Mean per Primer		14.8	10.6	

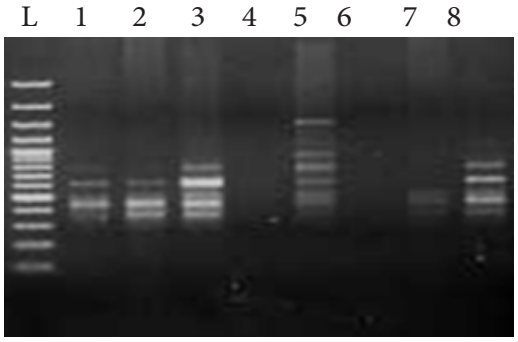
Similarity matrix of 8 genotypes generated by 15 primers

Genotypes	Matrix File Input							
	TT_1	TT_2	TT_3	TT_4	TT_5	TT_6	TT_7	TT_8
TT_1	1.00							
TT_2	0.64	1.00						
TT_3	0.40	0.39	1.00					
TT_4	0.00	0.00	0.00	1.00				
TT_5	0.28	0.27	0.39	0.00	1.00			
TT_6	0.00	0.00	0.00	0.99	0.00	1.00		
TT_7	0.28	0.26	0.16	0.00	0.32	0.00	1.00	
TT_8	0.31	0.41	0.26	0.00	0.48	0.00	0.38	1.00

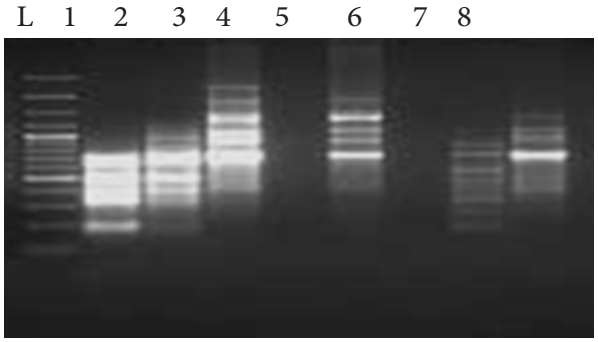
Genomic DNA isolation



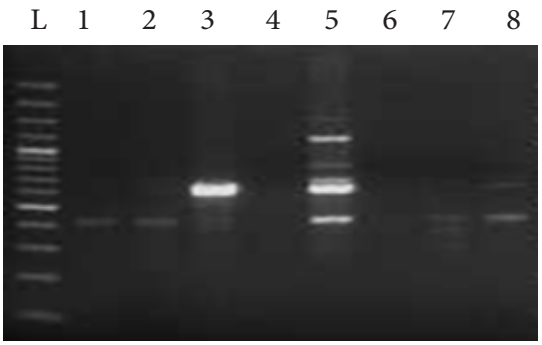
Quantification of 8 DNA samples: Lane L 1kb



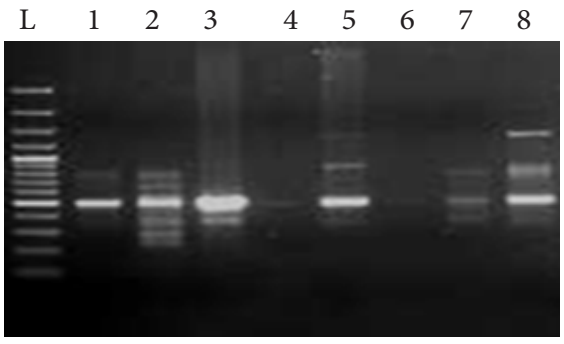
Primer-OPA 9



Primer-OPC 5



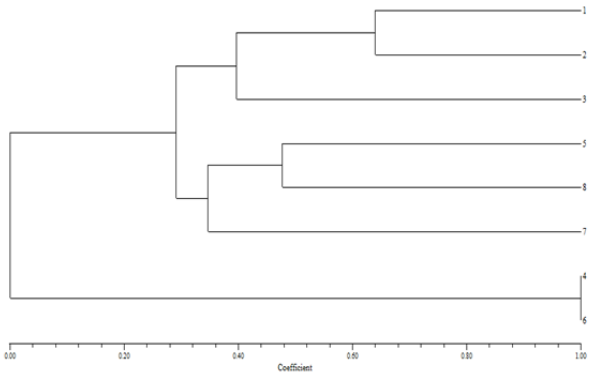
Primer-OPA 5



Primer-OPA 7

Polymorphic bands produce by different RAPD primers. DNA samples of *Tribulus terrestris* were collected from 8 different places of A.P. Lane L -100 bp DNA ladder, lane 1. Ahobilam, 2. Allagadda, 3. Banaganapalli 4. Mahanandi, 5 .Kovelakuntla, 6. R.K.Puram, 7. Uyyalawada, 8. Nandyal.

Dendrogram obtained from RAPD analysis using UPGMA



every random primers used in this technique. Where the results were under gone for band score and cluster analysis, 71.3% of polymorphism between the samples was observed. So that here we can clearly observe the genetic diversity from the collected samples even through from the same genus and family.

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