

Genetic Diversity Analysis of *Urtica Parviflora* in Uttarakhand Himalayas by Rapid Marker

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Abstract

Urtica parviflora is herbaceous perennial medicinal plant distributed in Himalayan region from 400m to 3000m amsl altitude range. In the present study, five genotypes of *U. parviflora* were collected from different altitudes having variability in morphological traits. This genotype was subjected to Random Amplified Polymorphic DNA (RAPD) marker analysis to assess the genetic diversity at molecular level and develop molecular marker for their identification. Fairly rich genetic diversity was found through RAPD marker analysis. A total 66 scorable bands were produced in five samples with 8 primers. Out of 66 bands, 43 bands were found to be polymorphic i.e., 65.15% polymorphism. The dendrogram of samples shows three major clusters. The samples of similar altitudes were found to be present in one cluster.

Keywords: *Urtica parviflora*; RAPD marker; Genetic diversity; Primer; Polymorphism

Introduction

Himalayan region harbors rich diversity of valuable medicinal plants, research should focused different levels for sustainable utilization of these resources in order to develop the medicinal plants sector [1].

Family Urticaceae (nettle family), a part of the larger group order Urticales, is mostly tropical and subtropical in both the hemispheres Zomlefer, Mabberley [2,3] have listed 48 genera with 1050 species in family Urticaceae. Nettles (Genus: *Urtica*), are herbaceous perennial plants but some are annual and few are shrubby. There are 30-45 species of flowering plant of the genus *Urtica* in the family Urticaceae, with a cosmopolitan though mainly temperate distribution. The most prominent member of the genus is the stinging nettles *Urtica parviflora* and *Urtica dioica*, native of Europe, Africa, Asia, and North America. The plant is evenly distributed in Himalayan region especially in lower to mid hill zone with 450m to 3500 m amsl altitudinal range [4]. *U. parviflora* leaves have stinging hairs and used in dysentery, joint pain and liver disorders [5]. Leaves and fresh roots used for the treatment of fractures, dislocation of bones, boils and treatment of dermatitis [6].

Knowledge of the amount and the distribution of genetic variation among the population provide a guide for the predicting the degree of inheritance, variation and level of heterosis that are prerequisite for effective breeding programs [7]. Genetic polymorphism is classically defined as the simultaneous occurrence of a trait in the same population of two or more discontinuous variants or genotypes. A major achievement occurred in marker technology with the development of markers based on DNA sequence. The ideal DNA marker should be highly polymorphic and evenly distributed throughout the genome. Co-dominant inheritance, easy access availability, high reproducibility is the properties of ideal DNA marker [8,9]. The genetic markers based on DNA provide a genetic diagnostic tool that permit direct identification of genotypes in environment independent manner in any tissue and developmental stage [10]. It will have great benefit for plant breeders when the DNA markers are closely linked to traits of interest. Various types of DNA-based molecular techniques were utilized to evaluate DNA polymorphism like RAPD, RFLP, AFLP [11-13]. The technique based on PCR amplification of genomic DNA with single primers of arbitrary nucleotide polymorphism was called Randomly Amplified

Polymorphic DNA (RAPD). It was reported that the percentage of GC contents of primers should be greater than 50% and primer sequence being 9-10 nucleotides in length [8,14]. The amplified fragments are separated electrophoretically and banding patterns are detected by different methods such as staining and autoradiography.

RAPD markers were used for evaluation of genetic diversity in many plant species such as tobacco [15] *Lycopersicon* [16], sugarcane [17], *Solanum* [18].

Materials and Methods

Collection of sample

Fresh juvenile leaves of *U. parviflora* were collected in the first week of month of March, 2011 from five different altitudes in Nainital district of Uttarakhand (India) viz., Mukteshwar (2800m amsl), Nainital (2200m amsl), Ghorakhaal (2000m amsl), Bhimtal (1300m amsl) and Haldwani (424 m amsl) [4]. These leaves were brought to laboratory in perforated polybags. After washing these were kept in deep freezer at -20°C temperature.

DNA extraction

DNA isolation was done using CTAB (Cetyl trimethyl ammonium bromide) method. The leaves were ground in liquid nitrogen and DNA was extracted with 2% CTAB extraction buffer (2% CTAB, 1.4M NaCl, 100 mM Tris HCl, 20mM EDTA and 1% Mercaptoethanol). Further isolation was continued according to the method described by Doyle and Doyle (1990) with some modifications due to high content of phenolic compounds and polysaccharides in *Urtica*. In the extraction buffer 2%-4% of PVP (Polyvinylpyrrolidone) was used for removal

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Received January 30, 2015; Accepted May 25, 2015; Published June 01, 2015

Citation: Sharma S, Ali SA, Khare A, Nailwal TK (2015) Genetic Diversity Analysis of *Urtica Parviflora* in Uttarakhand Himalayas by Rapid Marker. J Biotechnol Biomater 5: 183. doi:10.4172/2155-952X.1000183

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of these phenolic compounds. Phenol: chloroform: isoamylalcohol (25:24:1) was used in place of chloroform: isoamylalcohol (25:24). After addition of isopropanol, DNA gets precipitated along with pigments, due to high content of chlorophyll and polyphenols in this species. Therefore, immediately after adding isopropanol, DNA spooled out with the help of micropipette in separate eppendorf tube.

DNA quantification

Quality of the extracted DNA was checked by running on 0.8% agarose gel. Ethidium bromide solution was used to stain the gel. Purity and concentration of genomic DNA was estimated by calculating the ratio of optical densities measured at 260-280 nm with a spectrophotometer (Thermo Scientific Type UV1, England). Appropriate dilutions of DNA were made for further amplification and RAPD analysis.

Polymerase chain reaction

Polymerase chain reaction (PCR) was performed on DNA thermal cyclor (Biometra) using 20 µl reaction mixture containing 10mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM MgCl₂, 100 µM of each dNTP, 50pM Primer, 50 ng of DNA and two unit of *Taq* DNA polymerase. Twenty, Ten-base pair primers (GeNei™ Bangalore) were used for amplification reactions. Only those primers showing polymorphic results were selected for the purpose of diversity and similarity analysis. The reaction component was added to PCR tubes kept on ice. The PCR conditions of 94°C (1 minute) for denaturation, 35°C (1 minute) for annealing and 72°C (2 minutes) for primer extension were maintained.

PCR products were electrophoresed on 1.8% (w/v) agarose gels, in 0.5X TAE Buffer at 80 V for 3 hrs and then stained with ethidium bromide (0.5µg/ml). Gels with amplification fragments were visualized and photographed under UV light and the intensity of bands was observed. Out of twenty primers used eight primers produced recognizable bands. The photographs of gels were used to score data for RAPD markers. Each DNA fragment amplified by a given primer was considered as a unit character and the RAPD fragments were scored as present (1) or absent (0) for each of the primer accession combinations. Diversity coefficient of each primer (number of polymorphic bands/ total number of band) and pairs-wise genetic similarity were calculated using NTSYS-PC (version 2.11a) software [19].

Result and Discussion

The *Urtica* genotypes selected on the basis of morphological characters were subjected to DNA analysis to determine the genetic diversity available at different altitudes in Himalayan region. DNA isolated by CTAB (Cetyl trimethyl ammonium bromide) method yielded strong and reliable amplification products showing its compatibility for RAPD – PCR using random primers. DNA isolated by this method was of high purity (A_{260}/A_{280} ratio were 1.6-1.8) having very low contamination of polyphenols and polysaccharides (Table 1). For this purpose, DNA extracted from the *Urtica* genotypes was amplified using twenty random primers and run on the agarose gel. Out of 20 RAPD primers used, eight primers generated strong amplifications and resulted in polymorphic products (Table 2). The remaining primers were not considered for compiling the results because they were either not polymorphic or did not give clear amplifications. A total of 66 scorable bands were produced in five samples with eight primers. This data was utilized for further computation. Out of 66 bands 43 bands were polymorphic (65.15%). Data matrices were prepared in which the presence of bands was coded as (1) and absence as (0). The similarity index values obtained for pair wise comparison among *U.*

Sample	Altitudes (meters)	Absorbance			Quantity
		OD (260 nm)	OD (280 nm)	Ratio (260/280)	DNA (µg /µl.)
Mukteshwar	2800	0.052	0.031	1.7	0.65
Nainital	2200	0.182	0.096	1.8	2.27
Ghorakhal	2000	0.116	0.067	1.73	1.45
Bhimtal	1300	0.163	0.097	1.7	2.037
Haldwani	424	0.167	0.100	1.67	2.6

Table 1: The quantity and purity of DNA obtained from the qualitative and quantitative analysis of our five DNA samples.

S. No.	Primer (5N→3N)	Total number of loci	Number of monomorph loci	Number of polymorph loci	Polymorphism (%)
1.	CAGGCCCTTC	5	2	3	60.00
2.	TGCCGAGCTG	8	4	4	50.00
3.	CAATCGCCGT	7	2	5	71.42
4.	AGCCAGCGAA	9	4	5	55.55
5.	AGGGAACGAG	12	2	10	83.33
6.	GTTGCGATCC	8	3	5	62.50
7.	AGCGTCCTCC	11	4	7	63.63
8.	GGATGAGACC	6	2	4	66.66

Table 2: Polymorphism revealed by different RAPD primers.

Rows/Cols	Mukteshwar	Nainital	Ghorakhal	Bhimtal	Haldwani
Mukteshwar	J000E + 000				
Nainital	6.555555	1.0000000			
Ghorakhal	5.902439	5.750000	1.0000000		
Bhimtal	5.112121	4.944444	5.333333	1.0000000	
Haldwani	4.215151	4.578947	4.636363	7.857142	1.000000

Table 3: Jaccard's similarity analysis based on eight RAPD primers in five different altitudinal populations of *Urtica parviflora*.

parviflora populations from five different altitudes are shown in Table 3. The similarity index based on eight RAPD markers ranged from 42% to 78%. Similarity was found in genotype collected from similar altitudinal range pairs of Bhimtal and Haldwani (78%) order followed by Mukteshwar and Nainital (65%), Mukteshwar and Ghorakhaal (59%), Nainital and Ghorakhaal (57%) and Ghorakhaal and Bhimtal (53%). Minimum similarity was observed between the genotype pairs of Mukteshwar and Haldwani (42%). A dendrogram was constructed from five different populations of *U. parviflora* based on the similarity index (SI) values. The SI values were computed as a ratio of number of similar bands to total number in pair wise comparison of populations. The similarity matrix obtained by RAPD bands among five *U. parviflora* populations are shown in Table 3. Based on DNA finger-printing data, the genetic distances for five *U. parviflora* populations were calculated using NTSYS (Numerical Taxonomy System) program. Option Sequential Agglomerative Hierarchical Non-overlapping (SAHN) was used which resulted in the construction of dendrogram shown in Figures 1-4.

The dendrogram has grouped all the five populations into three major clusters A, B and C. Cluster A is further classified into two sub-clusters A1 and A2 and cluster B into sub clusters B1 and B2. The major clusters A and B are comprised of 2 populations, while the cluster C is comprised of one population. The maximum similarity was found between population A1 (Haldwani) and A2 (Bhimtal), with similarity index value 78%, while population C (Mukteshwar) and A1 (Haldwani) has shown least similarity i.e., 42%.



Figure 1: *U. parviflora* sample collected from five places of Kumaun region of Uttarakhand.

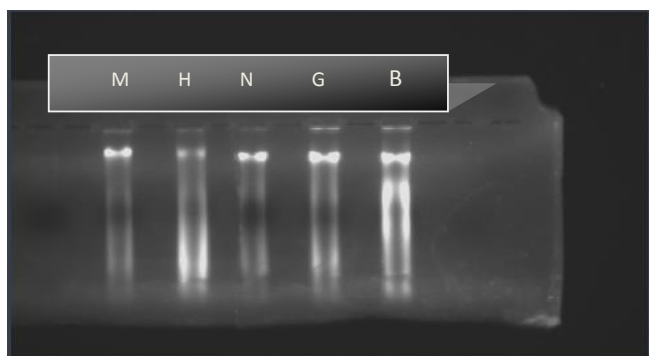


Figure 2: Agarose gel (0.8%) electrophoresis of genomic DNA extracted from *Urtica parviflora* by using Hi-Media plant DNA extraction kit based on CTAB method from five different altitudinal levels of Kumaun region

Molecular characterization of germplasm is basic to the improvement of the species and can be done at the DNA level. Present findings suggest that the genetic variability present in *U. parviflora* was quite appreciable due to elevational variation. As sample from lower altitudes (Haldwani) showed low genetic similarity with sample collected from higher altitudes (Mukteshwar). There is a general belief that nettle accessions found at higher elevations have high medicinal value compared to accessions found in lower altitudes. Various characters might be responsible for this assumption, as, *U. parviflora* leaves are known to be genetically variable in number of stinging hairs per unit leaf area as well as other characters. It was found that leaf samples collected from lower altitudes have lesser number of stinging hairs as compares to samples collected from higher altitudes. Overall variations in trichome numbers accounted for difference in important clinical values associated with this plant with respect to plant distribution at different altitudes. Various environmental factors like rainfall, temperature, altitude and the dosage of the UV rays may be responsible for such variation within the species. Spatial and genetic variations are often assumed to result from environmental heterogeneity and different selection pressure.

The results of study indicate that there is rich genetic diversity is available at molecular level in *U. parviflora* genotypes growing at different altitudes of Himalayan region in Uttarakhand. It would also be helpful for the planning of breeding strategies and future programs. The genetic diversity might be utilized for breeding purpose to tailor the genotypes having high medicinal value and useful for pharmaceutical industry.

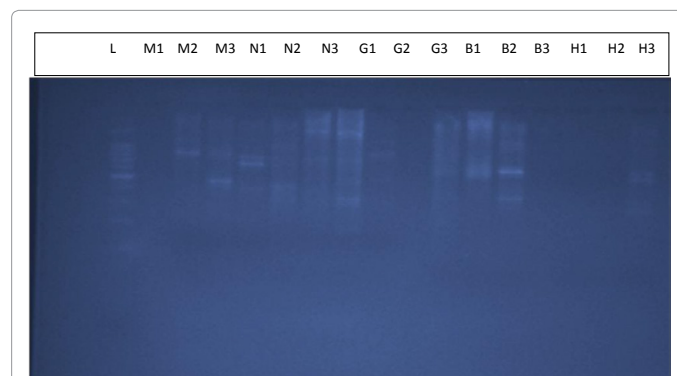


Figure 3: Amplified products were electrophoresed on a 1.8% agarose gel with 1X TAE Detected by staining with ethidium bromide (EtBr). Individual specific RAPD patterns of *U. parviflora* amplified by arbitrary primer (GGATGAGACC). Each lane shows different individual DNA samples. (M-Mukteshwar, N- Nainital, G-Ghorakhal, B- Bhimtal, H-Haldwani)

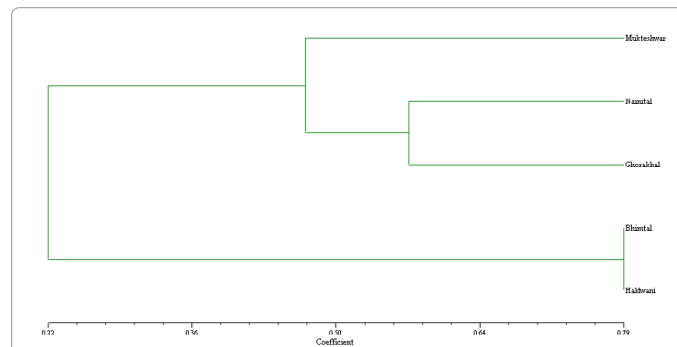


Figure 4: Neighbour-joining tree shows the relationship among different RAPD patterns Obtained in five populations of *U. parviflora* by using NTSYS program.

Acknowledgement

All experimental work completed in the biotechnology Lab., Departments of Biotechnology, Kumaun University, Nainital, Uttarakhand.

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