Research in Plant Biology, 4(2): 01-08, 2014 ISSN : 2231-5101
www.resplantbiol.com

Regular Article

Genetic diversity and relationships of selected *Polygonum* species using RAPD analysis

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The genetic diversity of the selected *Polygonum* species namely *P. chinense* L., *P. molle* D. Don, *P. glabrum* Willd., *P. barbatum* L., *P. fagopyrum* Moench. *P.hydropiper*, *P.nepalances*, was analyzed using RAPD markers. To analyze genetic structure of seven populations of *Polygonum* were sampled from its introduced regions in Southern Western Ghats. Out of 40 primers screened 11 generated distinct and reproducible DNA fragments, of which 49 (69%) were polymorphic and remaining 22 (31%) were monomorphic. Number of bands varied between 5 and 9 and average 6.5 bands per primer was observed. It indicates that the genetic diversity of *Polygonum* in Southern Western Ghats is extremely low, and all populations most likely consist of the same genotype. This study suggested that some other adaptability related factors other than the genetic diversity are responsible to the *Polygonum* rapid growth in India.

Keywords: Taxonomy, *Polygonum*, RAPD, Dendrogram, Phylogeny.

Polygonaceae Juss., the Buckwheat, Smartweed or Knotweed family and its cosmopolitan occurence, geographically distributed from the tropics to the arctic although most species are concentrated in the northern temperate region (Heywood, 1978). It is the family of dicots containing approximately 1,200 species in 48 genera. (Freeman and Reveal, 2005; Sanchez and Kron, 2008) Members of the Polygonaceae are diverse in habit ranging from annual or perennial herbs, shrubs to lianas and some trees.

The genus *Polygonum* L., is named Alafe-Haftband or Bandvash in Persian language and including nine endemic species (Mozaffarian, 1996; Rechinger, 1986). It is an important group of medicinal plants used for various purposes (Saeidnia *et al*., 2011). Literature review shows that the genus *Polygonum* is introducing as a source of numerous phenolic compounds, flavoids, anthraquinones, stilbenes and tannins (Lin *et al*., 2003). Among them, flavonoids are the most common compounds in *Polygonum* species and have previously been used as chemotaxonomic markers in the systematics of Polygonaceae plants (Datta *et al*., 2000).

The traditional taxonomic methods used for the identification of plants rely primarily on morphological observations or physiochemical methods, which cannot be used efficiently when only powdered forms

or shredded material is available (Young-Hum *et al*., 2003). When methods of DNA analysis including hybridization, amplification, fingerprinting and sequencing became widely available in the 1980's, scientists were quick to apply these sensitive techniques to the study of systematics, especially to the difficult cases where physical comparisons alone provided insufficient or conflicting data unsuitable for the construction of conclusive phylogenies.

The morphological markers constitute the main criteria of species distinction in taxonomy (Monika *et al*., 2006). Genetic analysis is now an indispensable tool for systematists in helping to differentiate between and declare new plant species, characterize the evolutionary relationships between lineages (Chris Brinegar, 2009). By using molecular techniques we have the possibility to validate the taxonomic classification, because the species-specific morphological differences should correspond to certain differences on the molecular level (Monika *et al*., 2006).

Recently, randomly amplified polymorphic DNA (RAPD) analysis has become a general method for estimating genetic diversity and variation among plant and cultivars (Ha *et al*., 2001; Oiki *et al*., 2001; Wang *et al*., 2001; Shaw, 1995) and also characterizing genetic polymorphisms since it does not require probe DNA and detailed information about the genomic and population polymorphism (Hartl, 1999). Furthermore, the RAPD technique has several advantages over restriction fragment length polymorphism (RFLP) analysis, namely speed, low cost and the ability to analyze small amounts of samples (Um *et al*., 2001; Tochila-Komatsu *et al*., 2001).

RAPD analysis has been used to investigate the genetic diversity and phlogenetic relationships among the populations in many cultivated plant species (Hormaza *et al*., 1994; Arias and Rieseberg, 1995; Bonnim *et al*., 1996; Mimura *et al*., 2000). The number of studies using DNA analysis to clarify evolutionary relationships and classify species has proliferated over the past 25 years (Chris Brinegar, 2009). In the present study, RAPD molecular markers were used to investigate the genetic diversity of *Polygonum* L. 15 populations from different regions in Southern Western Ghats were sampled and analyzed.

Materials and Methods

Plant sampling

Plant samples of five *Polygonum* species *viz*., *P. chinense , P. molle, P. glabrum, P. barbatum, P. fagopyrum. P.hydropiper, P.nepalances* was collected at flowering stage from different places of Nilgiri Biosphere Reserve (NBR), Southern India. The voucher specimens have been deposited (MH173546 to MH17352) at Madras Herbarium (MH), Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu, India. The details of the plant samples used in the study are presented in (Table 1).

DNA extraction

Total genomic DNA was isolated from young leaves by using DNeasy plant mini kit (Qiagen, Germany). The yield and purity were checked by electrophoresis in a 0.8% (w/v) agarose gel and also from the value obtained by UV absorbance ratio at 260 nm/280 nm.

RAPD assay

Random amplified polymorphic DNA (RAPD) analysis was performed according to the methodology of (Williams *et al.* 1990). Each amplification mixture of 25 µl contained 25 mM MgCl2, 10x *Taq* buffer, 100 µM of each dNTPs (Promega Corp., USA), and 0.3 mM of primer, 1.5 units of *Taq* polymerase (Promega Corp., USA) and 60 ng of template DNA. DNA amplification was performed in a Veriti 135 Well Thermal Cycler (Applied

Biosystems, USA) and RAPD-PCR was started at 94°C for 2 min, followed by 36 cycles of 94°C for 30 sec. 37°C for 1 min. 72°C for 2 min. and finally 72°C for 5 min. Amplification products were then separated by electrophoresis in 1.5% (w/v) agarose gels in 0.5X TBE buffer at 85 V. The gels were stained with ethidium bromide for 15 min and viewed under ultraviolet light with gel documentation.

Electrophoretic profiles were analyzed for polymorphism based on the presence and absence of DNA bands on agarose gel. Confirmation of results was done using at least three different samples of each species. Experiment was repeated at least three times to confirm species-specific fingerprinting pattern and reproducibility.

Table 1: Details of the plants sample used in the study

Plant Name	Origin (Place)	Latitude	Longitude	Altitude
Polygonum chinense L.	Kethi (P1a)	11°22′48.40″N	76°44′16.28″E	6864
	Burliyar (P1b)	11°20′56.01″N	76°49′38.40″E	3424
	Jagathala (P1c)	11°24′01.82″N	76°48′08.57″E	7301
P. glabrum Willd.	Ooty (P2a)	11°23′01.00″N	76°42′19.72″E	7012
	Naduvattam (P2b)	11°28′35.21″N	76°34′48.90″E	6807
	Kil Kundha (P2c)	11°17′26.83″N	76°38′42.82″E	6134
P. molle D.Don	Yedapalli (P3a)	11°22′24.86″N	76°49′03.04″E	6528
	Bandhalur (P3b)	11°28′42.75″N	76°19′57.89″E	3002
	Sholurmattam (P3c)	11°26′22.84″N	76°57′07.63″E	5534
P. barbatum L.	Kuntha, Ooty (P4a)	11°17′26.83″N	76°38′42.82″E	6134
	Ketti (P4b)	11°22′46.10″N	76°44′23.54″E	6925
	Burliyar (P4c)	11°20′15.20″N	76°49′57.09″E	3200
P. fagopyrum L.	Coonnur (P5a)	11°22′19.75″N	76°46′09.07″E	6311
	Nanjanad (P5b)	11°21′22.89″N	76°37′34.98″E	6880
	Devarsola (P5c)	11°32′57.79″N	76°27′06.46″E	3300
P.hydropiper L.	Coonnur (P6a)	11°21′51.24″N	76°45′31.05″E	6284
	Masinagudi (P6b)	11°33′58.39″N	76°38′13.50″E	3100
	Kil kotagiri (P6c)	11°26′41.45″N	76°56′38.96″E	5600
P.nepalances Meisn.	Ketti (P7a)	11°22′41.14″N	76°44′30.63″E	6819
	Naduvattam (P7b)	11°27′55.44″N	76°32′53.21″E	6475
	Coonnur (P7c)	11°23′01.40″N	76°44′01.61″E	7108

Genetic data analysis for RAPD

Amplified products, which were reproducible and consistent in performance, were scored for band presence (1) or absence (0) and a binary qualitative data matrix was constructed. Pair wise comparisons were calculated from the data matrix and analysis was carried out following UPGMA (unweighted pair-group method with arithmetic averages) method based on the Ward's minimum variance algorithm by using STATISTICA 8 (Marques de sa, 2007). Squared Euclidean Distances were calculated on the basis of the similarity coefficients. Percentage of polymorphic bands (PPB) was calculated by dividing the number of polymorphic bands by the total number of bands surveyed. The binary matrix was used to determine the genetic diversity, genetic differentiation and gene flow using the software POPGENE (Yeh *et al*., 1999) ver. 3.2. (Nei, 1973) gene diversity (*h*) using corrected allele frequency and the Shannon's index (I) were estimated within and among the populations. Average genetic diversity within population (H_S) and total genetic diversity among populations (H_T) were also calculated. Population differentiation was analyzed for polymorphism between populations by GST. Gene flow (Nm) was estimated from $Nm = 0.5 (1 - G_{ST})/G_{ST}$ (Nei, 1987). Analysis of molecular variance (AMOVA) (Excoffier *et al*., 1992) was used to calculate variation among and within population using GenAlEx (Peakall *et al*., 2006) ver. 6.41.

Results

A total of 71 clear and reproducible bands were amplified with different lengths, of which 49 (69%) were polymorphic and remaining 22 (31%) (Figure 1, Table 2) were monomorphic. Number of bands varied between 5 and 9 and average 6.5 bands per primer was observed. Maximum number of polymorphic bands (7) was noticed from OPP-12. The present amplification indicated that simple sequence repeats were abundant and highly dispersed throughout the genome of *Polygonum* populations. The amplified PCR fragment size ranged from 400 to 2200 bp (Fig 1)

The Nei's genetic diversity (*h*) and Shannon's indices (*I*) among all the population from 7 population were estimated as 0.1849 (SD=0.189) and 0.2478 (SD=0.287), respectively. Value for total genotype diversity among population (H_T) was 0.1574 while within population diversity (H_S) was found to be 0.0741(Table 3). The Mean coefficient of gene differentiation (G_{ST}) value 0.452 indicated a fairly high level of population differentiation. Estimate of gene flow (Nm) in the population was found as 0.2678, among the populations of 21 *Polygonum.* The population exhibited the high level of variability in *Polygonum molle* (h = 0.1668 ± 0.2164, I = 0.2420 ± 0.164) and *Polygonum chinense* population the lowest (h = 0.051 ± 0.092, I = 0.0121 ± 0.139). Percentage of polymorphic loci, h and I were also estimated in Southern Western Ghats (42.72%, 0.1736 and 0.2523, respectively). The high G_{ST} value in analyses clearly indicated high level of population differentiation. AMOVA result revealed highly significant genetic differences among the 21 populations. Of the total genetic diversity, 37% was attributable to among-populations diversity and the rest (63%) to differences within populations.

Discussion

The diversity of populations of a threatened species is essential for developing a conservation strategy. Genetic variability is the basis for adaptation and a species without enough genetic diversity is thought to be unable to cope with changing environmental conditions (Schaal *et al*., 1991). Nowadays,

Molecular marker can provide insights into the genetic divergence underlying morphologically indistinguishable taxa (Lee, 2000; Whittall *et al*., 2004; McKinnon *et al*., 2008). Previous analysis of RAPD markers Polymorphism detected in a population can be given in terms of the Nei's genetic diversity (h), Shannon's information index (I), total heterozygosity (HT), average heterozygosity (HS), coefficient of population differentiation (GST) and estimate of gene flow (Nm) (Zhao *et al*., 2000). The population genetics, a value of gene differentiation GST < 0.05, 0.05–0.15 and >0.15 classified as low, medium and high population differentiation respectively (Nei, 1978). In the present study mean coefficient of gene differentiation (G_{ST}) value 0.452 indicated a fairly high level of population differentiation. Estimate of gene flow (Nm) in the population was found as 0.2678 among the populations of *Polygonum*.

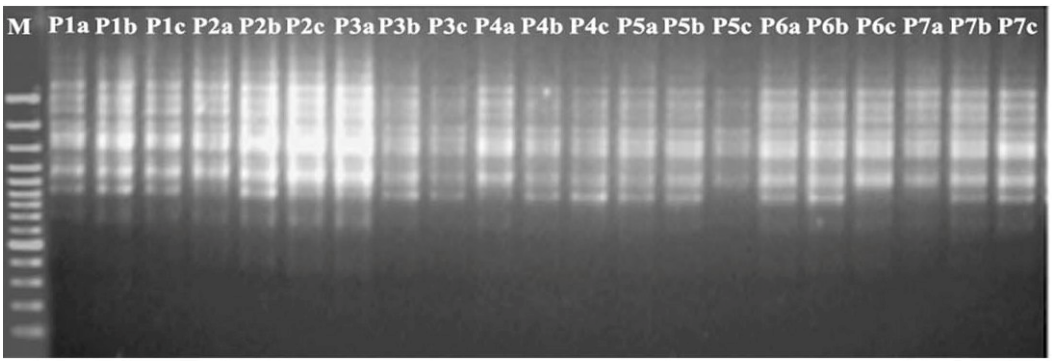

Fig. 1. The profile of RAPD products across 21 *Polygonum* spp. Amplified using primer (OPA-09) Lane p1a to P7c correspond to samples from the *Polygonum* spp. (accession codes as listed as table 1. M – DNA Marker

Table 2: Total number of amplified fragments and their polymorphic percentage generated using selected RAPD primers

Primer	Sequence (5′ to 3′)	Total bands	No. of Polymorphic Bands
OPA-05	5′-AGGGGTCTTG-3′	6	5
OPA-09	5′-GGGTAACGCC-3′	7	6
OPA-13	5′-CAGCACCCAC-3′	5	4
OPB-09	5′-TGGGGGACTC-3′	6	4
OPE-06	5′-AAGACCCCTC-3′	6	5
OPL-10	5′-TGGGAGATGG-3′	8	5
OPL-19	5′-GAGTGGTGAC-3′	6	4
OPA-14	5′-CAGCACCCAC-3′	5	3
OPP-11	5′-AACGCGTCGG-3′	9	5
OPP-12	5′-AAGGGCGAGT-3′	7	5
OPP-13	5′-GGAGTGCCTC-3′	6	3

Table 3: Genetic diversity indices *Polygonum* populations

Population	*H*	*I*	G_{ST}	*N*m	H_T	H_S
Seven Population analysis	0.1849 (0.189)	0.2478 (0.287)	0.452	0.2678	0.1574(0.0128)	0.741(0.0094)

*The values given in bracket are standard deviation.

The study concluded that the species of *Polygonum* examined in this study did not show high polymorphism, which may result from a high proportion of vegetative reproduction. The low level of polymorphism found in *Polygonum chinense* may be related to its geographical range limits. Further, the study suggested that the RAPD method is convenient and efficient for identifying the genotypes of plants from similar species. Also, these studies will enhance the creation of reference database for plant barcoding towards the ultimate goal of a digitalized encyclopedia of all plant species.

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