Bangladesh J. Bot. **39**(1): 97-102, 2010 (June)

USE OF RANDOM PCR (RAPD) TECHNOLOGY TO ANALYZE SYSTEMATIC RELATIONSHIPS IN TERRESTRIAL BLADDERWORTS (*UTRICULARIA* L.)

MD. OLIUR RAHMAN*

Laboratory of Plant Chromosome and Gene Stock, Graduate School of Science, Hiroshima University, Higashi Hiroshima, Japan

Key words: Utricularia, RAPD, PCR, Cluster analysis, Molecular taxonomy

Abstract

Polymerase Chain Reaction (PCR) based technology RAPD was tested for its applicability and suitability for molecular systematics of terrestrial bladderworts. PCR was carried out and the resultant products were subjected to agarose gel electrophoresis. The banding patterns were compared among ten species of *Utricularia*. Out of 40 random oligonucleotide primers examined, 16 primers generated distinguishable bands. Cluster analysis, unweighted pair group method with arithmetic mean (UPGMA) and ordination approach Scatter diagram using Matrix plot were performed based on RAPD fingerprints. The UPGMA and ordination analysis revealed a clear portrayal of systematic relationships in bladderwort species which were concordant with previous studies based on morphology and molecular approaches, indicating the reliability of RAPD markers for estimation of genetic variation and species relationships in *Utricularia*.

Introduction

Recent progress in DNA marker technology, particularly PCR based markers, such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), and inter simple sequence repeat markers (ISSR) have augmented the marker resources for genetic analyses of a wide variety of genomes. Among the PCR based technology the RAPD is a simple and easy method to detect polymorphisms based on the amplification of random DNA segments with single primer of arbitrary nucleotide sequence (Williams *et al*. 1990). Genomic DNA is subjected to the PCR, adding only a single short oligonucleotide of random sequence in each reaction. The method has many advantages over other kinds of DNA fragment analysis because it is inexpensive, fast and can be applied with limited amount of DNA, and is suitable for work on anonymous genomes (Hadrys *et al*. 1992). The RAPD method has been demonstrated to be useful for the studies of taxonomic problems (Vilatersana *et al.* 2005), systematic relationships (Pharmawati *et al.* 2004), phylogeny reconstruction (Poczail *et al.* 2008), population genetic structure (Sales *et al*. 2001), species hybridization (Caraway *et al.* 2001), assessment of genetic diversity (Souframanien and Gopalakrishna 2004), cultivar identification (Hu and Quiros 1991) and linkage mapping (Sondur *et al.* 1996).

The bladderworts (insectivorous genus *Utricularia*) are annual or perennial terrestrial, aquatic or epiphytic herbs, always found in damp places, without true roots or leaves but with stems modified in various ways to function as rhizoids, stolons and foliar organs. The majority of taxonomic studies on *Utricularia* have been carried out based on morphological characterization (Thor 1988, Taylor 1989). Pollen characters and chromosomal investigation have also been surveyed for potential use in *Utricularia* systematics (Huynh 1968, Casper and Manitz 1975). Recently some molecular markers including isozymes and ISSR markers were evaluated in *Utricularia* to detect genetic diversity and interspecific relationships (Araki 2000, Rahman and Kondo 2002, 2003a). Rahman (2007) applied some nuclear DNA markers for fingerprinting in the section *Utricularia*. Although random amplified polymormhic DNA markers were employed in some aquatic species of *Utricularia*, these markers have never been tested in terrestrial species (Rahman 2006). The use of random PCR (RAPD) technology, as a tool in genetic characterization and study of genetic variation among bladderworts will help to elucidate the variation found

*Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh. E-mail: dr_oliur@yahoo.com

within the genus *Utricularia*. In the present study PCR based molecular markers RAPDs were employed to study the genetic variability among different terrestrial bladderwort species and to determine the systematic relationships among them.

Materials and Methods

Plant materials: Ten terrestrial bladderworts *viz.*, *Utricularia alpina* Jacq., *U. amethystina* Salzm. *ex* St. Hill, *U. bifida* L., *U. caerulea* L., *U. calycifida* Benj., *U. humboldtii* Schomb., *U. longifoila* Gardner, *U. praelonga* A. St. Hill & Girard, *U. triflora* P. Taylor and *U. uliginosa* Vahl were used in this study.

Genomic DNA extraction: DNA was extracted from leaves of fresh materials by Cetyl Trimethyl Ammonium Bromide (CTAB) method with slight modification (Rahman and Kondo 2003a). Approximately 1.0 - 1.8 g of leaf material was ground to a fine powder in liquid nitrogen with a mortar and pestle before isolation of DNA. The isolated DNA was dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA).

PCR and electrophoresis: PCR was carried out using 10-mer oligonucleotide primers. The RAPD primers used in this study are shown in Table 1. PCR amplification was carried out in 12µl reaction mixture containing 20 ng of genomic DNA, 10 pmol of primer, 1 µl X10 Taq buffer, 1 µl of dNTP mixture, 0.05 µl Taq polymerase enzyme, and deionised water to a final volume of 12 µl. Amplification was carried out in a PTC-100 thermal cycler programmed for 45 cycles with

Table 1. 10-mer oligonucleotides used as primers in the RAPD assay.

Primer	Sequence	% GC content	Primer	Sequence	% GC content
OPA1	5′-CAG GCC CTTC-3′	70	OPB4	5′-GGA CTG GAG T-3′	60
OPA2	5′-TGC CGA GCT G-3′	70	OPB5	5′-TGC GCC CTT C-3′	70
OPA3	5′-AGT CAG CCA C-3′	60	OPB6	5′-TGC TCT GCC C-3′	70
OPA4	5′-AAT CGG GCT G-3′	60	OPB8	5′-GTC CAC ACG G-3′	70
OPA7	5′-GAA ACG GGT G-3′	60	OPB11	5′-GTA GAC CCG T-3′	60
OPA9	5′-GGG TAA CGC C-3′	70	OPB15	5′-GGA GGG TGT T-3′	60
OPA13	5′-CAG CAC CCA C-3′	70	OPB17	5′-AGG GAA CGA G-3′	60
OPB1	5′-GTT TCG CTC C-3′	60	OPB18	5′-CCA CAG CAG T-3′	60

the following temperature profile: denaturation at 94º C for 1 min, annealing at 35º C for 1 min and extension at 72º C for 2 min. Cycling was concluded with a final extension at 72º C for 5 min. PCR products were electrophoresed in 1.5% agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA), visualized by ethidium bromide staining and photographed under UV light.

Data analysis: Electrophoretic data were scored for the presence (1) or absence (0) of PCR fragments. Rogers and Tanimoto's coefficient was employed to detect genetic affinity among the species examined. UPGMA (Unweighted pair group method with arithmetic mean) tree was constructed by clustering the dissimilarity data based on Dist coefficient using SIMINT (Similarity for Interval data) program. A scatter diagram was constructed to view the clustering of the taxa using MXPLOT (Plots 2-way scatter diagrams of rows or columns of a matrix) where the distance matrix was transformed using the DCENTER (Performs a 'double-centering' of a matrix of similarities or dissimilarities among the objects) and MDSCALE (Multidimensional scaling analysis) options. All analyses were performed with the NTSYS-pc package, version 2.1 (Rohlf 2000).

Results and Discussion

RAPD-PCR were performed in ten terrestrial bladderworts to assess genetic variability and to infer systematic relationships. Sixteen RAPD markers generated robust and reproducible bands among the *Utricularia* species examined. All reactions were duplicated and only highly reproducible bands were considered. *Utricularia uliginosa* presented the highest number of amplified bands while *U. alpina* generated the least number of fingerprints.

Across the bladderwort species studied the genetic similarity index was calculated based on Rogers and Tanimoto's coefficient. The estimates of similarity index in *Utricularia* species as revealed by RAPD-PCR is presented in Table 2. The interspecific genetic similarity indices ranged from 0.27 between *U. uliginosa* and *U. longifolia* to 0.87 between *U. alpina* and *U. longifolia* indicating that these two species (*U. alpina* and *U. longifolia*) are most closely related. The same similarity (0.87) has also been observed between *U. triflora* and *U. amethystina* showing their close relationship. In other species this similarity index varied from 0.33 to 0.75 (Table 2).

Table 2. Similarity matrix among ten terrestrial bladderworts (*Utricularia* L.) using the Rogers and Tanimoto's coefficient based on RAPD fragments.

Species	*U. alpina*	*U. amethystina*	*U. bifida*	*U. caerulea*	*U. calycifida*	*U. humboldtii*	*U. longifolia*	*U. praelonga*	*U. triflora*	*U. uliginosa*
U. alpina	1									
U. amethystina	0.65	1								
U. bifida	0.47	0.55	1							
U. caerulea	0.47	0.55	0.55	1						
U. calycifida	0.65	0.55	0.55	0.55	1					
U. humboldtii	0.65	0.55	0.75	0.55	0.75	1				
U. longifolia	0.87	0.55	0.40	0.40	0.55	0.55	1			
U. praelonga	0.65	0.55	0.55	0.55	0.75	0.75	0.55	1		
U. triflora	0.55	0.87	0.47	0.47	0.47	0.47	0.47	0.47	1	
U. uliginosa	0.33	0.40	0.40	0.75	0.40	0.40	0.27	0.40	0.33	1

Cluster analysis of dissimilarity estimates was performed to construct a UPGMA tree showing overall genetic relationship among *Utricularia* species (Fig. 1). The UPGMA dendrogram constructed from RAPD data revealed that *Utricularia* species could be placed in two main groups.

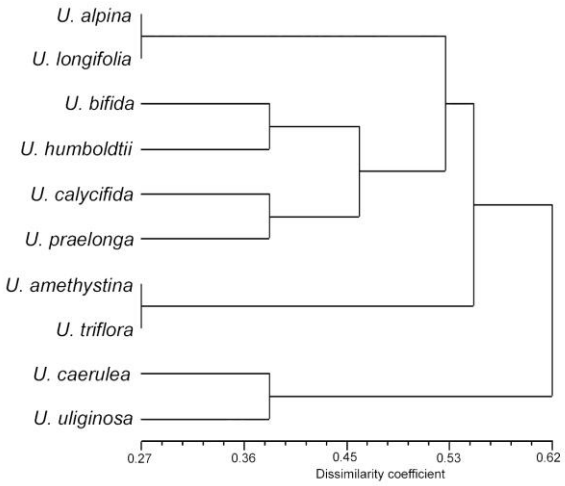

Fig. 1. UPGMA tree illustrating relationships among the terrestrial bladderworts (*Utricularia* L.) studied by RAPD markers.

The first group consisted of *U. alpina, U. longifolia, U. bifida, U. humboldtii, U. calycifida, U. praelonga, U. amethystina* and *U. triflora*; while the second group was formed by *U. caerulea* and *U. uliginosa*. The lowest dissimilarity was detected between *U. alpina* and *U. longifolia* as well as between *U. amethystina* and *U. triflora* suggesting that *U. alpina* is most closely allied to *U. longifolia* while *U. amethystina* to *U. triflora* as they grouped together (Fig. 1).

An ordination approach scatter diagram was carried out to view the clustering of the species. The result of the scatter diagram based on Matrix Plot is shown in Fig. 2. The ordination approach based on RAPD profiles resulted in placing the species into two clusters. The first cluster composed of *U. caerulea* and *U. uliginosa* whereas the remaining eight species formed the second cluster. Results obtained from the cluster method (UPGMA) and ordination method (Scatter diagram) presented the same pattern of species relationships in *Utricularia*.

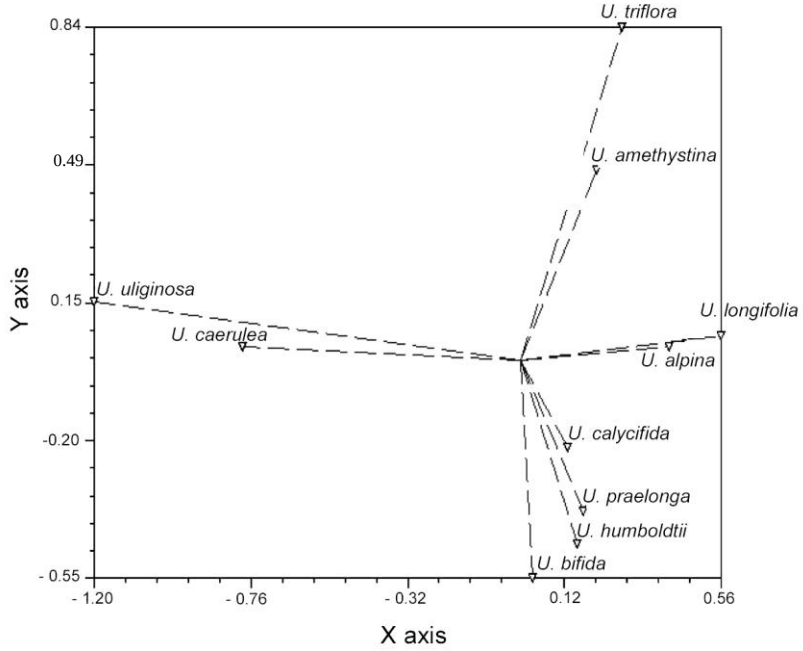

Fig. 2. Scatter diagram showing the clustering pattern of the terrestrial bladderworts (*Utricularia* L.) based on RAPD fragments.

A number of different approaches using primers of arbitrary sequence in polymerase chain reaction have been developed to analyse genetic variation (Williams *et al.* 1990). RAPD provides an effective alternative to RFLP (Waugh and Powell 1992). The RAPD methodology is flexible because by changing the amplification conditions, a single primer can conveniently amplify from a few to numerous different sequences to produce amplified fingerprints (Caetano-Anollés *et al.* 1991). Though RAPD method is subjected to criticism, for example, casting doubt on its correctness because of the appearance of artifact bands, careful optimization of the RAPD protocol has been shown to increase the reproducibility of the RAPD data (Micheli *et al.* 1994).

In the present study PCR-based RAPD markers were used to assess genetic relatedness in terrestrial bladderworts and they have been found potential to characterize *Utricularia* species. In a previous study Rahman (2006) found RAPD markers suitable to detect genetic variation and species relationships in aquatic bladderworts (*Utricularia*). Among the aquatic species *U. australis* is most closely related to *U. dimorphantha*, and *U. bremii* with *U. gibba*. *Utricularia aurea* was

found distantly related to *U. bremii* (Rahman, 2006). In contrast, the present study revealed that there is a high genetic affinity between *U. alpina* and *U. longifolia* indicating that these two species are most closely related to each other across the terrestrial bladderworts. Using inter simple sequence repeat (ISSR) markers Rahman and Kondo (2003a) showed that these two species are very close to each other. Morphologically, *U. alpina* and *U. longifolia* show a close relationship by sharing the following characteristic: leaves elliptic or obovate, calyx-lobes ovate to ovate-deltoid, filaments curved and ovary ovoid (Taylor 1989). The present study also revealed that *U. bifida* grouped with *U. humboldtii* showing that they are closely allied (Fig. 1). Based on dissimilarity indices and UPGMA clustering pattern the present study confirms the findings of a previous study conducted by Rahman and Kondo (2003b). On the basis of pollen characters Sohma (1975) showed that both in *U. caerulea* and *U. uliginosa* pollen grains are mostly tricolporate, rarely oblate-spheroidal in equatorial view, circular in polar view, furrows meridinal and exine tectate. A close relationship was also detected between these species in the present study. Therefore, the results obtained from the random PCR technology have been found congruent with findings generated from morphological and other molecular data indicating that RAPD markers provide an accurate portrayal of relationships in *Utricularia*.

Acknowledgement

The author is grateful to Prof. Katsuhiko Kondo of the Hiroshima University, Japan for his cooperation during this study.

References

Araki S. 2000. Isozyme differentiation between two infraspecies taxa of *Utricularia australis* R. Br. (Lentibulariaceae) in Japan. Acta Phytotax. Geobot. **51**(1): 31-36.

Caetano-Anollés G., B.J. Bassam and P.M. Gresshoff. 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Biotech. **9**: 553-557.

Caraway V., G.D. Carr and C.W. Morden. 2001. Assessment of hybridization and introgression in lava-colonizing Hawaiian *Dubautia* (Asteraceae: Madiinae) using RAPD markers. Am. J. Bot. **88**: 1688-1694.

Casper S.J. and H. Manitz. 1975. Beitrage zur taxonomie und chorologie der mitteleuropäischer *Utricularia*–Arten, 2. Androsporogenese, chromosomenzahlen und pollenmorphologie. Feddes Repert. **86**: 211-232.

Hadrys H., M. Balick and B. Schierwater. 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol. Ecol. **1**: 55-63.

Hu J. and C.F. Quiros. 1991. Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Report **10**: 505-511.

Huynh K.L. 1968. Etude de la Morphologie du pollen du genre *Utricularia* L. Pollen et Spores **10**: 11-55.

Micheli M.R., R. Bova, E. Pascale and E. D'Ambrosio. 1994. Reproducible DNA fingerprinting with the random amplified polymorphic DNA (RAPD) method. Nucleic Acids Res. **22**: 1921-1922.

Pharmawati M., G. Yan and I.J. McFarlane. 2004. Application of RAPD and ISSR markers to analyse molecular relationships in *Grevillea* (Proteaceae). Austr. Syst. Bot. **17**: 49-61.

Poczail P., J. Taller and I. Szabo. 2008. Analysis of phylogenetic relationships in the genus *Solanum* (Solanaceae) as revealed by RAPD markers. Plant Syst. Evol. **275**: 59-67.

Rahman M.O. 2006. Evaluation of RAPD markers for taxonomic relationships in some aquatic species of *Utricularia* L. (Lentibulariaceae). Bangladesh J. Plant Taxon. **13**(2): 73-82.

Rahman M.O. 2007. DNA fingerprinting in *Utricularia* L. section *Utricularia* as revealed by PCR based assay. International J. Bot. **3**(1): 56-63.

Rahman M.O and K. Kondo. 2002. Evaluation of inter simple sequence repeat (ISSR) for systematic relationship of some terrestrial species of *Utricularia* L. (Lentibulariaceae). pp. 175-188. Proc. 4th Int. Carnivor. Plant Conf., Tokyo.

Rahman M.O. and K. Kondo. 2003a. Genetic diversity and species relationship of some terrestrial bladderwort (*Utricularia* L.) as revealed by inter simple sequence repeat (ISSR) markers. Acta Phytotaxon. Geobot. **54**(1): 49-57.

Rahman M.O. and K. Kondo. 2003b. Molecular characterization of some closely related species of *Utricularia* L. using inter-SSR markers. Bangladesh J. Plant Taxon. **10**(2): 11-19.

Rohlf F.J. 2000. NTSYSpc: Numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software, Setauket, New York.

Sales E., S.G. Nebauer, M. Mus and J. Segura. 2001. Population genetic study in the Balearic endemic plant species *Digitalis minor* (Scrophulariaceae) using RAPD markers. Am. J. Bot. **88**: 1750-159.

Sohma K. 1975. Pollen morphology of the Japanese species of *Utricularia* L. and *Pinguicula* L. with notes on fossil pollen of *Utricularia* from Japan (2). J. Jap. Bot. **50**(7): 193-208.

Sondur S.N., R.M. Manshardt and J.I. Stiles. 1996. A genetic linkage map of papaya based on randomly amplified polymorphic DNA markers. Theor. Appl. Genet. **93**: 547-553.

Souframanien J. and T. Gopalakrishna. 2004. A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. Theor. Appl. Genet. **109**: 1687-1693.

Taylor P. 1989. The genus *Utricularia* - a taxonomic monograph. Kew Bulletin, Additional Series XIV. Her Majesty's Stationery Office, London. 724 pp.

Thor G. 1988. The genus *Utricularia* in the Nordic countries, with special emphasis on *U. stygia* and *U. ochroleuca*. Nordic J. Bot. **8**: 213-225.

Vilatersana R., T. Garnatje, A. Susanna and N. Garcia-Jacas. 2005. Taxonomic problems in *Carthamus* (Asteraceae): RAPD markers and sectional classification. Bot. J. Linn. Soc. **147**: 375-383.

Waugh R. and W. Powell. 1992. Using RAPD markers for crop improvement. Trends in Biotech. **10**: 186-191.

Williams J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**: 6531-6535.

(Manuscript received on 10 February 2010; revised on 19 April 2010)