Tropical Plant Research
An International Journal
© Society for Tropical Plant Research

ISSN (E): 2349 – 1183
ISSN (P): 2349 – 9265
2(1): 10–16, 2015

Research article

A study of genetic diversity in *Oryza rhizomatis* D.A. Vaughan accessions using AFLP markers and morphological traits

G. Rajkumar[1,2]*, O. V. D. S. J. Weerasena[2] and K. K. S. Fernando[3]

[1]Department of Botany, Faculty of Science, University of Jaffna, Sri Lanka
[2]Institute of Biochemistry Molecular Biology and Biotechnology, University of Colombo, Sri Lanka
[3]Agricultural Biotechnology Centre, Faculty of Agriculture, University of Peredeniya, Sri Lanka

*Corresponding Author: gowrir@jfn.ac.lk & gowri450@yahoo.com [Accepted: 03 February 2015]

Abstract: *Oryza rhizomatis* is a wild rice species endemic to Sri Lanka and is reported to have tolerance to biotic and abiotic stresses prevailing in the dry zone of the country. Several morphological differences have been observed in *O. rhizomatis* grown under different agro-ecological conditions of Sri Lanka. Therefore, these rice species (accessions) may have genetic differences to consider them as 'ecotypes'. Morphological and genetic diversity of *O.rhizomatis* accessions collected from different locations were carried out to observe genetic differences among them. Scattered diagram of PCoA of *O. rhizomatis* accessions, based on morphological data showed that there were no significant differences among them. According to molecular analysis by AFLP, all the accessions were genetically similar and Jaccard genetic similarity coefficient varied with a very narrow range between the accessions (0.915 to 1.000). According to the results, the *O. rhizomatis* accessions tested did not show significant morphological and genetic differences. In this study representative samples from different locations were grown in the green house**,** exposing them to the same environmental conditions. This may have contributed to results obtained with no observable morphological differences. Further studies have to be carried out with *insitu* collections from different locations when extreme weather conditions are prevailing to see the morphological characters are changed as an adaptation and to assess any genetic diversity.

Keywords: AFLP - *Oryza rhizomatis* - Polymorphism - PCoA.

[**Cite as:** Rajkumar G, Weerasena OVDSJ and Fernando KKS (2015) A study of genetic diversity in *Oryza rhizomatis* D.A. Vaughan accessions using AFLP markers and morphological traits. *Tropical Plant Research* 2(1): 10–16]

INTRODUCTION

Oryza rhizomatis is an endemic wild rice species of Sri Lanka and is reported to have tolerance to biotic and abiotic stresses. *O. rhizomatis* plants are grown under different agro-ecological conditions of the island. This wild rice species studied is growing under different agro-ecological conditions of the island, especially drought, high temperature (Hambantoda, Anuradhapura), and high salinity areas (Puttalam and Ampara). *O. rhizomatis* species may have morphological, biochemical, anatomical etc. adaptations for their survival in the particular environmental conditions.

Sri Lankan researchers have found several morphological differences among accessions of *O. rhizomatis* species collected from different locations of Sri Lanka. There are no documentary evidences to support these eco-morphological differences. Therefore morphological and molecular comparisons of *O. rhizomatis* accessions collected from different locations were carried out by using forty five Morphological Descriptors (qualitative and quantitative) and Amplified Fragment Length Polymorphism (AFLP) analysis of DNA followed by cluster analysis.

MATERIAL AND METHODS

Morphological studies of Oryza rhizomatis accessions

Seeds from seven *Oryza rhizomatis* accessions which have been collected from different locations of Sri Lanka (Table 1). Seeds were planted in equal diameter pots filled with soil collected from paddy field with five

Received: 17 November 2014 Published online: 28 February 2015

replications for each accession, watered daily and grown under greenhouse conditions at the Plant Genetic Resources Centre, Gannoruwa, Peredeniya.

Table 1. List of *Oryza rhizomatis* accessions and their locations used in this study.

Accession	Location
WR-AC-85	Inginimitiya (Kurunegala)
WR-AC-20	Anuradhapura
WR-AC-08	Illakattuwa (Puttalam)
WR-AC-51	Tabbowa (Puttalam)
WR-AC-49	Kalladiya (Puttalam)
WR-AC-50	Samagipura (Puttalam)
WR-AC-28	Thambiyiwa (Anuradhapura)
WR-AC-03	Aukana (Anuradhapura)

Note: WR-AC – Wild Rice Accession.

Forty five morphological descriptors (both qualitative and quantitative) were used for characterization with vegetative and seed parameters of primary importance. Evaluations were performed as described for rice plant by the International Rice Research Institute (IRRI) using the scale established for each descriptor and data were recorded. Mean values for different morphological traits and standard errors were calculated from mean values of each accession by SPSS version 14 software (SPSS, Inc., Chicago IL). Principal components analysis was performed using MVSP 3.1 (Kovach 1998).

AFLP analysis of Oryza rhizomatis accessions collected from different locations

Extraction of DNA

Genomic DNA was extracted from rice leaves using CTAB based method as described by Chen *et al*. (1999). The extracted DNA was quantified by Agarose gel electrophoresis. Then concentrations of all DNA samples were adjusted to 300 ng.µl^{-1}.

AFLP analysis

AFLP analysis was performed as described by Vos *et al*. (1995). Briefly; DNA from each sample was digested with *Eco*R1 and 5 units of *Mse*1 enzymes. The digestion sample was incubated at 37^{o}C for 3.5 hours. To the double digested DNA, *Eco*R1 adapter (10 pmol.µl^{-1}), *Mse*1 adapter (10 pmol.µl^{-1}), 5U of T4 DNA ligase (New England Biolabs, USA), 10 mM ATP (GE Healthcare Life Sciences, UK), and 1X RL buffer were added and incubated overnight (~16 h) at 37^{o}C in a water bath. Then 3.0 µl of ligated samples were run on 1.5% agarose gel and visualized under the UV transilluminator to check the ligation.

After the ligation of adapters, 2 µl of digested/ligated DNA were preamplified in 25 µl of reaction containing 20 pmol of each preamplification primers, 0.5 mM dNTPs, 1U of Taq DNA polymerase (Genscript USA), 1X PCR buffer containing 1.5 mM $MgCl_2$ (Genscript USA) and sterile water. The PCR amplification was performed for 30 cycles of denaturation at 94^{o}C for 30 sec, annealing at 56^{o}C for 30 sec, extension at 72^{o}C for 60 sec in thermal cycler (Eppendorf ® Master cycler gradient). Then 3.0 µl of selective amplified samples were run on 1.5% agarose gel and visualized under the UV transilluminator to check the amplification. The preamplification product was diluted 20 times with sterile distilled water and used as a template for selective amplification.

The selective amplification reaction was conducted in final volume of 20 µl containing diluted preamplification product, fluorescently labeled *Eco*R1 primer, *Mse*1 primer, dNTPs, Taq DNA polymerase, PCR buffer. Then PCR amplification was carried out. The amplified samples were purified by ethanol precipitation followed by washing with 70% ethanol. The dried pellets were re-suspended in water and mixed with ET550-ROX size and deionized formamide. Then the samples were denatured at 95^{o}C for 2 minutes and analyzed by capillary electrophoresis on automated MegaBACE 1000 DNA sequencer. AFLP fragment analysis was performed with Genetic Profiler software.

Data analysis

Peaks in the electropherogram were analyzed and compared by using MegaBACE Genetic Profiler software. Jaccard's similarity coefficients (Jaccard 1901) were calculated using binary data and similarity coefficient matrix was generated to assess the genetic resemblances among varieties. Then the similarity matrix was used

for cluster analysis by Unweighed Pair Group Method with Arithmetic mean (UPGMA) method and the dendrogram was generated. The confidence of the UPGMA clusters were assessed by Mantel test (Mantel 1967) to calculate the cophenetic correlation coefficient (*r*). Principal Component Analysis (PCoA) was performed to find out possible relations that could not be visualized in cluster analysis.

RESULTS

Morphological studies of Oryza rhizomatis accessions

Eight accessions of *O. rhizomatis* species collected from different location of Sri Lanka were differentiated using thirty nine morphological traits. Significant variation was not observed for fourteen qualitative parameters; ligule colour, collar colour, auricle colour, internode colour, culm strength, secondary branching, panicle exsertion, panicle shattering, panicle threshability, awing, apiculus colour, stigma colour, lemma and

Table 2. Means and standard errors for the quantitative traits for eight accessions of *Oryza rhizomatis*.

Descriptor (Quantitative traits)	Mean ± SE
Seedling height(mm)	62.90±1.843
Leaf blade length(mm)	34.02±1.382
Leaf blade length width (cm)	0.985±0.0321
Ligule length (mm)	2.960±0.0814
Days to heading	62.00±0.737
Culm length (cm)	62.63±0.894
Culm number	4.90±0.178
Culm diameter (mm)	4.68±0.145
Panicle Length (cm)	22.783±0.2773
100 grain weight (g)	1.250±0.0268
Grain length (mm)	6.212±0.1562
Grain width (mm)	2.190±0.0395
Maturity (days)	110.37±1.296

Table 3. Scales, means and standard errors for the qualitative traits for eight accessions of *Oryza rhizomatis*.

Descriptor (qualitative traits)	Scale	Mean ± SE
leaf blade pubescence	1-3 (1=glabrous, 3=intermediate)	1.87±0.096
leaf blade colour	1-7 (1=pale green, 7=purple)	2.25±0.106
Basal leaf sheath colour	1-4 (1=green, 4=purple)	1.75±0. 069
leaf angle	1-4 (1=erect, 4=descending)	2.12±0.053
flag leaf angle	1-4 (1=erect, 4=descending)	3.38±0. .265
Ligule colour	0-3 (1=absent, 3=purple)	1.00±0.000
Ligule shape	0-3 (1=absent, 3=turnate)	1.62±0. 078
Collar colour	1-3 (1=pale green, 3=purple)	1.00±0.000
Auricle colour	1-3 (1=absent, 3=purple)	1.00±0.000
Culm angle	1-8 (1=erect, 8=procumbent)	4.50±0..340
Internode colour	1-4 (1=green, 4=purple)	1.00±0.000
Culm strength	1-9 (1=strong, 9=very weak)	1.00±0.000
Panicle type	1-9 (1=compact, 9=open)	7.50±0.310
Secondary branching	0-3 (0=absent, 3=clustering)	1.00±0.000
Panicle exsertion	1-8 (1=well exserted 8=enclosed)	1.00±0.000
Panicle axis	1-2 (1=straight, 2=droopy)	1.38±.078
Panicle shaterring	1-5 (1=very low, 5=high)	1.00±0.000
Panicle threshability	1-3 (1=difficult, 3=easy)	1.00±0.000
Apiculus colour	1-7 (1=whitet, 7=purple apex)	3.00±0.000
Stigma colour	1-4 (1=white, 5=purple)	3.00±0.000
Lemma and Palea colour	0=10 (0=straw, 10=white)	9.00±0.000
Lemma and Palea pubescence	1-5 (1=glabrous, 5=long hairs)	3.38±0.078
Sterile lemma colour	1-4 (1=straw, 4=purple)	1.75±0.208
Sterile lemma length (mm)	1-9 (1=short, 9=asymmetrical)	2.670±0.0723
Spikelet sterility	1-9 (1=highly fertile, 9=completely sterile)	3.75±0.155
Seed coat colour	1-7 (1=white, 7=purple)	4.00±0.000

palea colour and seed coat colour. Other qualitative traits also showed less variation among the accessions: Leaf blade pubescence varied from glabrous to intermediate. Leaf blade colour varied from pale green to dark green. Similarly quantitative traits showed little variations among the accessions. Means and standard errors for the quantitative traits are listed in table 2. Means, standard errors, scales for each qualitative traits are listed in table 3. There were no significant differences as evident from standard error (SE).

Multivariate Principal Coordinate Analysis (PCoA) of the morphological data indicated that the first, second and third components accounted for 53.461, 18.442 and 13.810% of the variance among accessions, respectively. Thus, the first three components explained 85.713% of variance.

Both qualitative and quantitative characters were considered and PCoA was performed. The scattered diagram of PCoA of eight *O. rhizomatis* accessions with five replicates based on morphological data obtained is showed in figure 1.The most important parameters separating accessions in the first component were grain length (r =-0.758) and sterile lemma length (r = -0.654) and were negatively correlated with the first component. Culm length, culm diameter, and hundred grain weight were also negatively correlated with the first component, while other traits were positively correlated with the first component.

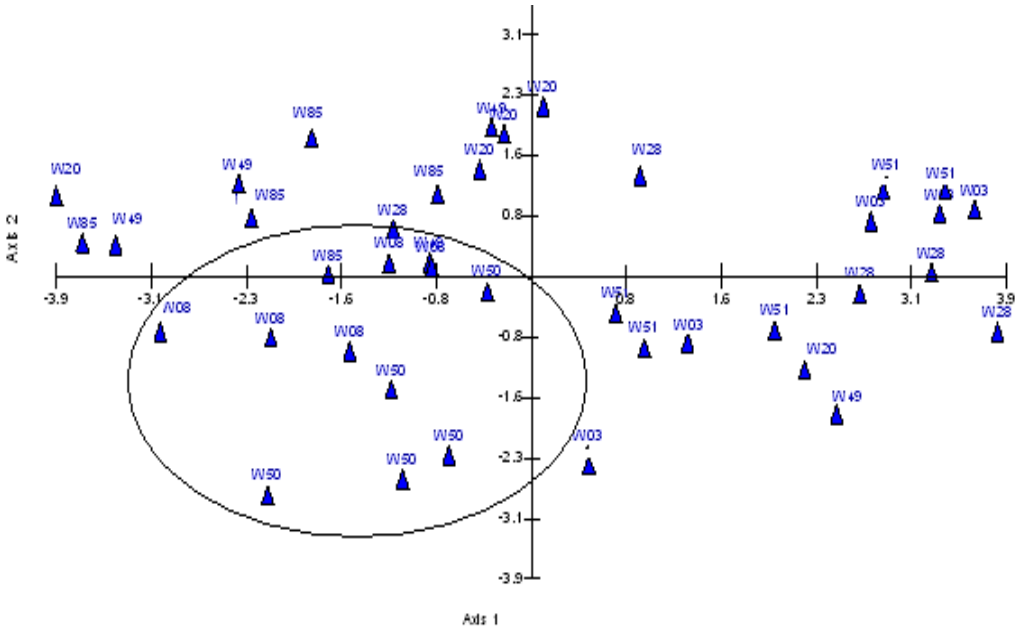

Figure 1. Two dimensional PCoA plot of relationships among *Oryza rhizomatis* accessions based on thirty nine morphological parameters. Ovale indicate the well- defined group.

Grain width had the highest correlation with the second component (r = 0.805) while the other traits: Seedling height, leaf blade length, and width, Culm length, panicle Length, grain length were also positively correlated. Other quantitative characters showed negative correlation with the second components. Replicates of accession W08 and W50 were grouped together (indicated in oval). But replicates of other accessions did not group together. All replicates were scattered randomly.

AFLP analysis of Oryza rhizomatis accessions collected from different locations

Polymorphism

Six pairs of primers generated a total of 93 fragments. Of these only 13 fragments were polymorphic (13.9%) and 80 (86.1%) were monomorphic. The fragments ranged in size from 30 to 550 base pairs. The number of amplified products generated by individual pair of primer ranged from 13 (E-AA ×M-G) to 18 (E-AT × M-G) with an average of 15.5 fragments per pair of primers.

Genetic relationship by cluster analysis

All fragments (93) scored were used for genetic diversity studies. The results obtained by the Jaccard's similarity coefficients showed that the genetic similarity varied with a narrow range from 0.915 to 1.000 (Table 4). The accession R-AC-28 and R-AC-49, R-AC-08 and R-AC-20 showed the lowest genetic similarity (0.915) while the accessions R-AC-08 and R-AC-50 showed the highest genetic similarity (1.000). Similarity matrix

generated for all *O. rhizomatis* accessions showed less genetic variation among the accessions. The genetic similarity 0.930 was used to establish a cut off value for cluster generation. At this cut off value in UPGMA analysis separated *O. rhizomatis* accessions into two main clusters (Fig. 2) I and II. Cluster I contained 3 accessions while cluster II enclosed 5 accessions.

Table 4. Similarity matrix based on Gower general similarity coefficient for *Oryza rhizomatis* accessions.

	R-AC-50	R-AC-49	R-AC-28	R-AC-85	R-AC-20	R-AC-51	R-AC-08	R-AC-03
R-AC-50	1.000							
R-AC-49	0.957	1.000						
R-AC-28	0.957	0.915	1.000					
R-AC-85	0.936	0.936	0.936	1.000				
R-AC-20	0.915	0.915	0.936	0.936	1.000			
R-AC-51	0.968	0.947	0.968	0.947	0.926	1.000		
R-AC-08	1.000	0.957	0.957	0.936	0.915	0.968	1.000	
R-AC-03	0.936	0.936	0.979	0.936	0.957	0.947	0.936	1.000

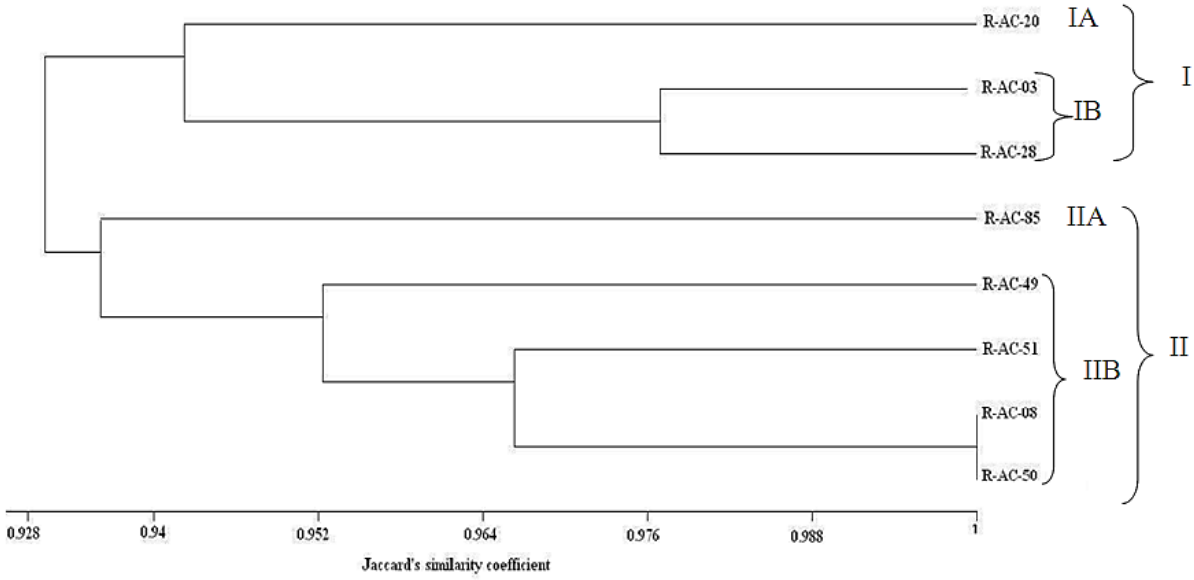

Figure 2. UPGMA dendrogram showing genetic similarity among *Oryza rhizomatis* accessions based on Jaccard's similarity coefficient.

The cluster I again subdivided into two as IA and IB at the similarity coefficient of 0.928. Cluster IA encloses only one accession R-AC-20 and cluster IB encloses two accessions R-AC-03 and R-AC-28. Cluster II subdivided into two clusters IIA and IIB at the similarity coefficient 0.934. Cluster IIA encloses only one accession R-AC-85 while cluster IIB subdivided into two clusters at the similarity coefficient 0.952. Accessions R-AC-08 and R-AC-50 originated from the same node and having highest genetic similarity (1.000).

Principal Coordinate Analysis (PCoA) of 8 *O. rhizomatis* accessions based on AFLP data obtained from the similarity matrix constructed by Gower general coefficient is showed in figure 3. The distribution of groups produced by PCoA analysis confirmed the clustering pattern of the UPGMA analysis. The accession R-AC 50 and R-AC-08 were found at the same position in the scatter diagram as both having 1.000 similarity coefficients.

DISCUSSION

Oryza rhizomatis plants are grown under different agro-ecological conditions of the island. This wild rice species has got adapted to climatic conditions prevailing in the Hambantota, Kurunegala, Puttalam, Anuradhapura, Monaragala, and Ampara districts of Sri Lanka (Liyanage 2010). Sri Lankan researchers have found several morphological differences among accessions of *O. rhizomatis* species collected from different locations of Sri Lanka. There are no documentary evidences to support these eco-morphological differences.

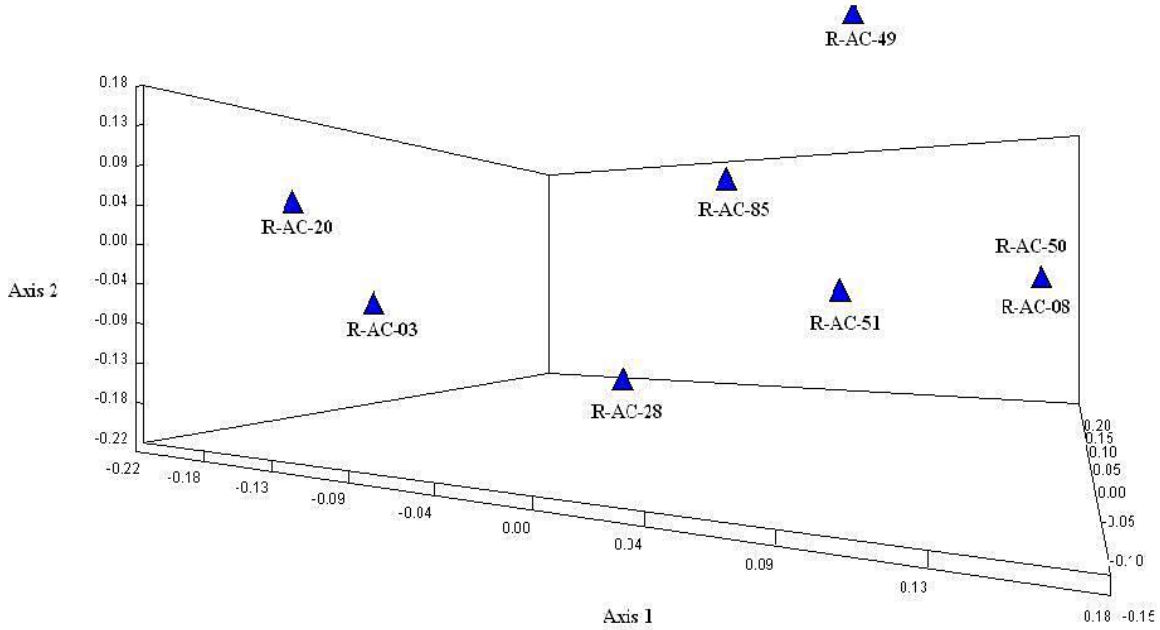

Figure 3. Scatter diagram of first three principal cordinates (PCo1, PCo2 and PCo3) of *Oryza rhizomatis* accessions.

This wild rice species studied is growing under different agro-ecological conditions of the island, especially drought, high temperature (Hambantoda, Anuradhapura), and high salinity areas (Puttalam and Ampara). *O. rhizomatis* species may have morphological, biochemical, anatomical etc adaptations for their survival in the particular environmental conditions. However, according to our results, there are no prominent morphological differences observed among the accessions collected from different locations of Sri Lanka. Scattered diagram (Fig. 1) of PCoA of eight *O. rhizomatis* accessions developed based on morphological data showed that there are no significant differences among the accessions. However, accession number 50 and 08 were grouped together indicating the closeness of these two accessions (indicated as oval in the figure 1). While replicates of other accessions were scattered among them indicating their morphological dissimilarity. Therefore it is not possible to arrive of a firm conclusion that the accessions collected from different locations are morphologically similar. Further studies have to be carried out using collections from different locations when extreme weather conditions are prevailing to see the consistency of morphological characters.

According to the molecular analysis all the accessions were genetically similar because Jaccard genetic similarity coefficient varies with a very narrow range from 0.915 to 1.000 between the accessions (Table 4). R-AC-08 and R-AC-50 showed the highest genetic similarity (1.000). These two accessions were originated from the same node of the UPGMA dendrogram (Fig. 2) and placed in the same position in the scattered diagram (Fig. 3). Similarly the replicates of these two accessions were grouped together in the scattered diagram generated for morphological analysis (Fig. 1). These results indicate that those two accessions were morphologically and genetically similar. These two accessions were collected from the same area, Puttalam. However some other two accessions collected from the Puttalam area (AC-51 and AC-49) did not group together with previous (AC-08 and AC-50) in the scattered diagram of morphological analysis (Fig. 1). Results of AFLP analysis of these four accessions AC-08, AC-50, AC-51 and AC-49 were clustered together in cluster IIB in the UPGMA dendrogram (Fig. 2) constructed for the molecular analysis and indicated that all four are genetically similar. It is suggested to repeat the analysis with more replicates from each location to confirm the results.

CONCLUSION

The morphological differences of the accessions collected from different locations (Liyanage, Personal communication) may be due to climatic differences prevailed at the time of collections. In this study representative samples from different locations were grown under greenhouse conditions, common for all the accessions. This may have contributed to results with no observable morphological differences. Further studies

have to be carried out with *in situ* collections from different locations when extreme weather conditions are prevailing to see the morphological characters are changed as an adaptation and to assess any genetic diversity.

ACKNOWLEDGEMENTS

The authors greatly appreciate the financial support given by the National Research Council of Sri Lanka (Grant No. 05-61).

REFERENCES

Chen DH & Ronald PC (1999) A Rapid Minipreparation Method Suitable for AFLP and PCR Applications. *Plant Molecular Biology Reporter* 17: 53–57.

Jaccard P (1901) Étude comparative de la distribuition floraledans une portion des Alpes et des Jura. *Bulletin de la Societe vaudoise des sciences naturelles* 37: 547–579.

Kovach WL (1998) MVSP - A Multivariate Statistical Package for Windows, ver. 3.1. Kovach Computing Services, Pentraeth, Wales, U.K.

Liyanage ASU (2010) Eco-geographic survey of crop wild relatives, Plant Genetic Resources Centre, Gannoruwa, Peredeniya, Sri Lanka.

Mantel NA (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research* 27: 209–220.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J & Kuiper M (1995) AFLP: a new technique for DNA fingerprinting. *Nuclic Acids Research* 23: 4407–4414.