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Genetic diversity analysis in South African taro (*Colocasia esculenta*) accessions using molecular tools

Mwamba Nguluta[1], Patrick Adebola[2] and Michael Pillay[1]*

[1]Departtment of Biotechnology, Vaal University of Technology, Private Bag X021, South Africa.
[2]Africa Rice Center (AfricaRice), Abidjan, Cote d'Ivoire.

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Taro [*Colocasia esculenta* (L) Schott] belongs to the family Araceae. It is an important staple food crop grown mainly by small scale farmers in many parts of the world. Taro is grown in South Africa from the coastal parts of the northern Eastern Cape to the KwaZulu-Natal north coast. Although it is an important staple in South Africa, very little information exists on the genetic diversity of the crop. Knowledge of the genetic diversity of a crop is important for breeding programmes. The aim of this study is to assess the genetic diversity of taro using ITS2 sequencing and assess whether the ITS2 secondary structures could be used as a taxonomic marker to group the taro accessions. Currently, taro accessions in South Africa have not been placed into any type of groups and the accessions are named from the locality where they are collected. The ITS2 sequence data separated the accessions into 4 clusters. The accessions did not group according to geographical locations. The ITS2 secondary structure had one common motif present in all 25 accessions suggesting that it could be used as a taxonomic marker for taro. Other motifs were able to place taro accessions into groups. The discovery of these motifs strengthens the potential of the ITS2 secondary structure as taxonomic marker in taro. The high genetic diversity provides taro breeders a selection of parents for the improvement of taro.

Key word: Root crop, genetic diversity, internal transcribed spacer2.

INTRODUCTION

Colocasia esculenta (taro) is a vegetatively propagated root crop in the family Araceae (Kreike et al., 2004). The family contains about 100 genera and 1500 species that are mainly distributed in the tropical and subtropical regions of the world (Wang and Higa, 1983). Taro is an important staple food crop for millions of people in developing countries in Asia, Africa and Central America (Sharma et al., 2008). The most important food source from the plant is the root (corms and cormels). However, the leaves, leaf stalks, and petioles are also used as a vegetable (Shange, 2004). Taro is rich in carbohydrates, proteins, minerals and vitamins. Taro is also used as a medicinal plant for the treatment of tuberculosis, ulcers, pulmonary congestion and fungal infections in some countries. In South Africa, taro is known as amadumbe which refers to the swollen underground stem. Taro is cultivated mainly in the subtropical coastal area starting at Bizana district in the Eastern Cape and includes the

*Corresponding author. E-mail: mpillay@vut.ac.za.

rest of coastal KwaZulu-Natal. There is less cultivation of the crop in the midlands and generally none in the northern parts of the province where the climate is drier and cooler (Shange, 2004).

In spite of the plant's commercial value, few studies have been conducted to genetically improve the crop (Sharma et al., 2008). The taxonomy and nomenclature of taro is not well established and cultivars are generally named from the place where they are collected. Taro exhibits a wide range of morphological variation both qualitatively and quantitatively (Trimanto et al., 2010). Although a wealth of genetic resources exist, attempts to conserve the germplasm of taro and use it to solve production problems has not been successful. An assessment of the genetic variation within taro is a prerequisite for initiating efficient breeding programmes for obtaining desirable genotypes (Kreike et al., 2004).

Taro germplasm characterization using molecular methods contributes to the knowledge of genetic relationships between accessions and therefore facilitates the breeding of taro cultivars to satisfy market needs and responses to diverse biotic (e.g., taro leaf blight) and abiotic (e.g., drought and salinity) challenges (Soltis et al., 1992). The objective of this study was to determine the genetic diversity of taro by assessing the sequence variation of the Internal Transcribed (ITS) Spacer 2 regions. Furthermore, this study also predicted the ITS2 secondary structures of the taro accessions to ascertain its value as a taxonomic marker for the crop. This information may be useful in establishing the identity of the taro accessions in South Africa for breeding purposes since the accessions are only known from the locations in which they are collected. South African taro germplasm have not been allocated to any morphological or molecular taxonomic groups.

MATERIALS AND METHODS

The 25 accessions used in this study were obtained from the germplasm bank at the Agricultural Research Council-Vegetable and Ornamental Plant Institute (ARC-VOPI) in Roodeplaat. The institute originally collected the plant material from different locations in South Africa. The accessions are named from the locality in which they were collected. Three plants of each accession were regrown in the greenhouse at the Vaal University of Technology.

PCR amplification and sequencing of the ITS2 region

DNA was extracted from young taro leaves according to the CTAB method (Saghai-Maroof et al., 1984). Components for 25 µl PCR reactions were: 100 ng of DNA, 10 pmoles each of ITS3 (GCATCGATGAAGAACGCAGC), and ITS4 (TCCTCCGCTTATTGATATGC) (White et al., 1990), 25 mM $MgCl_2$, (dGTP, dCTP, dATP and dTTP), and 0.2 µl GoTaq® polymerase 9.8 µl distilled water, 1x amplification buffer, 0.1 mM of each dNTP(Promega, Madison, WI). The amplification conditions for PCR were as follows: 40 cycles, each cycle consisting of a denaturation step at 94°C for 1 min, an annealing step at 50°C for 1 min and an extension step at 72°C for 1 min 30 s. After the 40th cycle, a final extension step was performed at 72°C for 7 min. The amplification reactions were performed in a Bio-Rad C1000 Thermo Cycler™ (Bio-Rad, Hercules, CA). After amplification, the products were separated by electrophoresis on a 1% agarose gel immersed in TBE buffer (90 mM Tris-borate, 2 mM EDTA, pH 8.0) to determine the size of the amplicons. Following amplification, the products were sequenced at Inqaba Biotech (Pretoria, South Africa).

ITS2 sequences analysis

The sequence chromatograms were visualized and edited using Chromas Lite 2.1.1 (Technelysium Pty, Brisbane, Australia). The sequences were aligned using ClustalW (Larkin et al., 2007) and appropriate cut-offs were applied.

ITS2 dendrogram analysis

The taxonomic relationships among the 25 taro sequences were determined using the Molecular Evolutionary Genetics Analysis version 5 software (MEGA5) (Tamura et al., 2011). The dendrogram was generated from the ITS2 sequence data, using the neighbour-joining method (Saitou and Nei, 1987).

Secondary structure prediction using ITS2 sequences

Secondary structures were derived from the ITS2 sequences by using the Sfold web server. Sfold predicts secondary structures based on statistical sampling paradigm to fold nucleic acids (http://sfold.wadsworth.org/cgi-bin/sirna.pl) (Ding and Lawrence, 2003). Default settings were used to derive the models.

RESULTS AND DISCUSSION

ITS2 sequence analysis and dendrogram

Amplification of the internal transcribed spacer (ITS2) of the rDNA of taro produced a fragment of approximately 450 bp in all the accessions. However, sequencing of the fragment showed that the length of the ITS2 region ranged from 399 bp in Mangozi to 406 bp in Tshwane 3. The G~C content of the ITS2 region ranged from 62.2 to 67.8% while the A~T content varied from 32.3 to 36.9%. The aligned sequence data for the ITS2 region had 403 nucleotides. Among these, 160 were variable, 242 were conserved and 127 were informative. The latter was used for parsimony analysis. The cluster analysis generated with the neighbour joining method (Saitou and Nei 1993) from the ITS2 sequences of taro is shown in Figure 1.

The clustering based on the ITS2 sequences generated a tree that was divided into four groups. With the exception of parts of groups II and IV, the dendrogram (Figure 1) derived from the ITS2 sequence data did not group the accessions according to their locality. The close affinity of accessions Tshwane 1 and Tshwane 2 (group II) is explanable by the fact that they accesions are not identical. A similar reason may be extended for the accessions from Maphumulo and Empangeni in group III. It was interesting to find that the

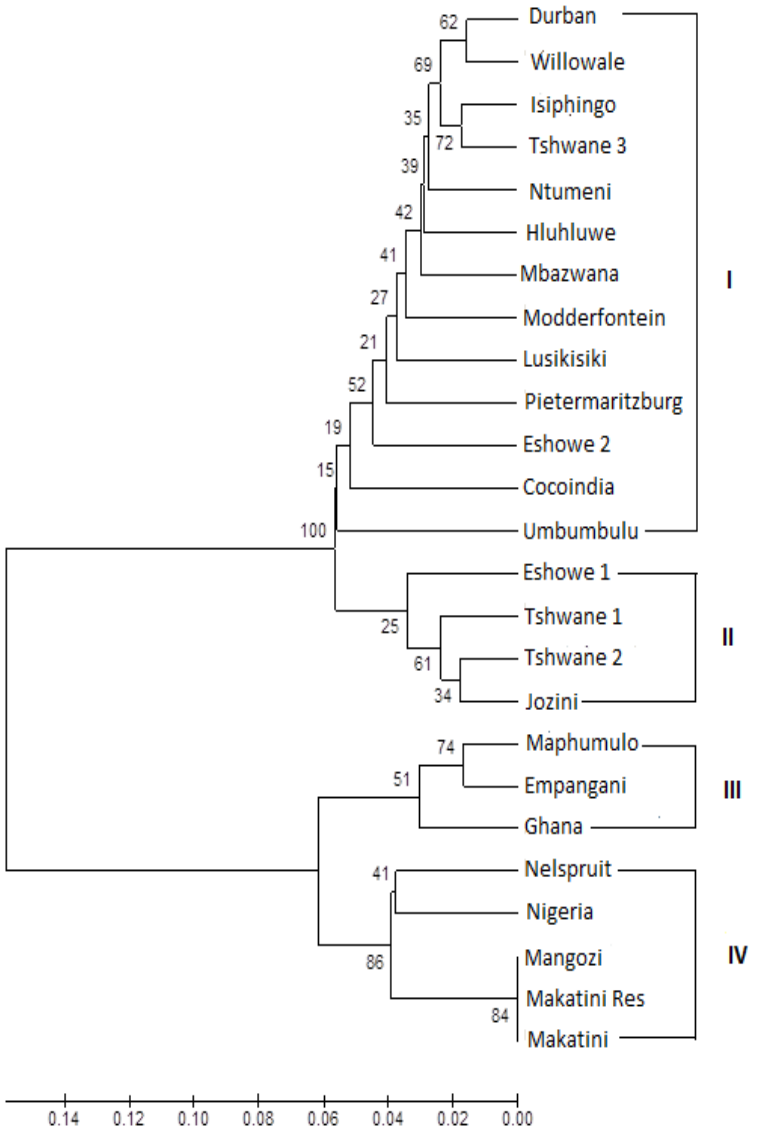

Figure 1. Dendrogram derived from ITS2 sequence data showing relationships between taro accessions. The names in the dendrogram represent the locality from which the accessions were collected.

accesion from Nigeria with a purple stem shared a sister relationship with that from Nelspruit that had a purple leaf. The close grouping of the accessions from Makatini, Makatini Res and Mangozi may be explained by the proximity of these locations and the transfer of material from one place to another by farmers. These accessions are identical.

Secondary structure of ITS2

The secondary structures of the ITS2 region were analysed based on variable and conserved elements in all 25 accessions of taro. The closeall 25 accessions of taro. The 25 accessions had an average of seven helices, seven hairpins, three bulge loops, three multi-branch loop, eight interior loops, and a dangling end (Table 1).

In general, these structures conform to the proposed four helices ITS2 model for flowering plants and green algae (Mai and Coleman, 1997), yeast and vertebrates(Joseph et al., 1999), Scleractinian corals (Chen et al., 2004), Lepidteran species (Kuracha et al., 2006), *Anopheles culicifacies* (Dassanayake et al., 2008) and eukaryotes as a whole (Schultz et al., 2005). However, this study showed that most of the accessions had from 5 to 11 additional helices. Similar finding were reported for corals (Chen et al., 2004), and in bivalve subfamily Pectininae (Salvi et al., 2010). On the contrary, in cnidarians various helices were found to be missing (Oliveiro et al., 2009). It was suggested that closely related taxa with very similar primary sequences can

Table 1. The ITS2 secondary structure element counts in taro.

Accessions	Hairpin	Helix	Bulge loop	Multi-branch loop	Interior loop	Dangling end	Energy
Durban	3	3	5	1	1	1	-83.7
Empangeni	7	8	2	1	4	1	-157.09
Eshowe 1	10	7	1	1	11	1	-140.3
Eshowe 2	8	8	2	3	7	1	-166
Ghana	9	9	3	2	2	1	-154.76
Hluhluwe	6	6	5	2	11	1	-163.1
Isipingo	8	6	1	2	10	0	-103.52
Jozini	5	6	2	2	8	1	-173.7
Lusikisiki	8	7	4	4	9	1	-175.8
Makatini	8	9	4	2	10	1	-169.1
Mangozi	9	8	1	2	9	1	-162.5
Maphumulo	6	6	4	1	7	1	-165.6
Mbazwana	9	9	1	5	12	1	-206.8
Modderfontein	8	8	5	4	5	1	-110.88
Nelspruit	8	8	6	2	9	1	-156.4
Nigeria	6	8	8	8	6	1	-142.4
Ntumeni	6	7	1	3	12	1	-145.2
Pietermaritzburg	7	10	2	3	12	1	-165.76
Tshwane 1	8	5	4	3	8	1	-128.3
Tshwane 2	5	6	3	3	10	0	-115.87
Tshwane 3	10	11	3	4	8	1	-138.4
Umbumbulu	7	7	4	4	12	1	-166.8
Willowvale	6	6	2	1	6	1	-67.64
Cocoindia	10	8	1	3	11	0	-162.2
Makatini Res	8	8	1	1	8	1	-159.8

result in/exhibit different structures (Hershkovitz and Zimmer, 1996). One of the distinct hallmarks of the core secondary structure is the highly conserved motif of 5' - UGGU- 3' and the deviation 5'- UGG- 3' found in all eukaryotes. This was also found in the predicted structures of taro at approximately nucleotide positions (291-293).

The predicted secondary structures of the ITS2 among the 25 taro accessions were quite variable and did not exhibit a common model based on topology. However, there was a common motif in all the accessions 5'-GCCCGAGGCCACUAGGCCGAGGGC-3' at approximately nucleotide positions (48-73) within helix 1 of the secondary structures; an example is shown from the sample Maphumulo in Figure 2. This motif may be a useful taxonomic marker for the identification of the species *C. esculenta.*

This is the first study to identify and report such a recurring motif in taro. This motif conserved in all the accessions is probably due to a functional significance of the region. Van Nues et al. (1995) suggested that the nature of the conserved motifs may probably have a function in the regulation of the transcription of active ribosomal subunits as this provides structural elements necessary for the correct pre-rRNA processing. Besides the common motif found in all accessions at positions (48-73), other motifs were identified in some accessions (Figure 3). Table 2 contains a list of accessions that exhibit the motifs shown in Figure 3a to g. These motifs may be useful in placing the taro accessions into groups based on their molecular characteristics. Prior to this study there has been no grouping of taro accessions in South Africa. To an extent, some of these groups corresponded to the grouping of the accessions based on the ITS2 sequence data (Figure 1). Further research is necessary to determine if these groups share similar agronomic traits. In that case, the groupings may be useful for breeders wishing to genetically improve taro.

Conclusion

The ITS2 secondary structure has been used for genusand species identification (Chen et al., 2010). The discovery of a common motif in helix 1 of the ITS2 secondary structure of all the taro accessions suggests that it may represent a molecular marker for identification of the species *C. esculenta*. This is the first study that has predicted the ITS2 secondary structures in taro. The presence of certain common motifs in some of the

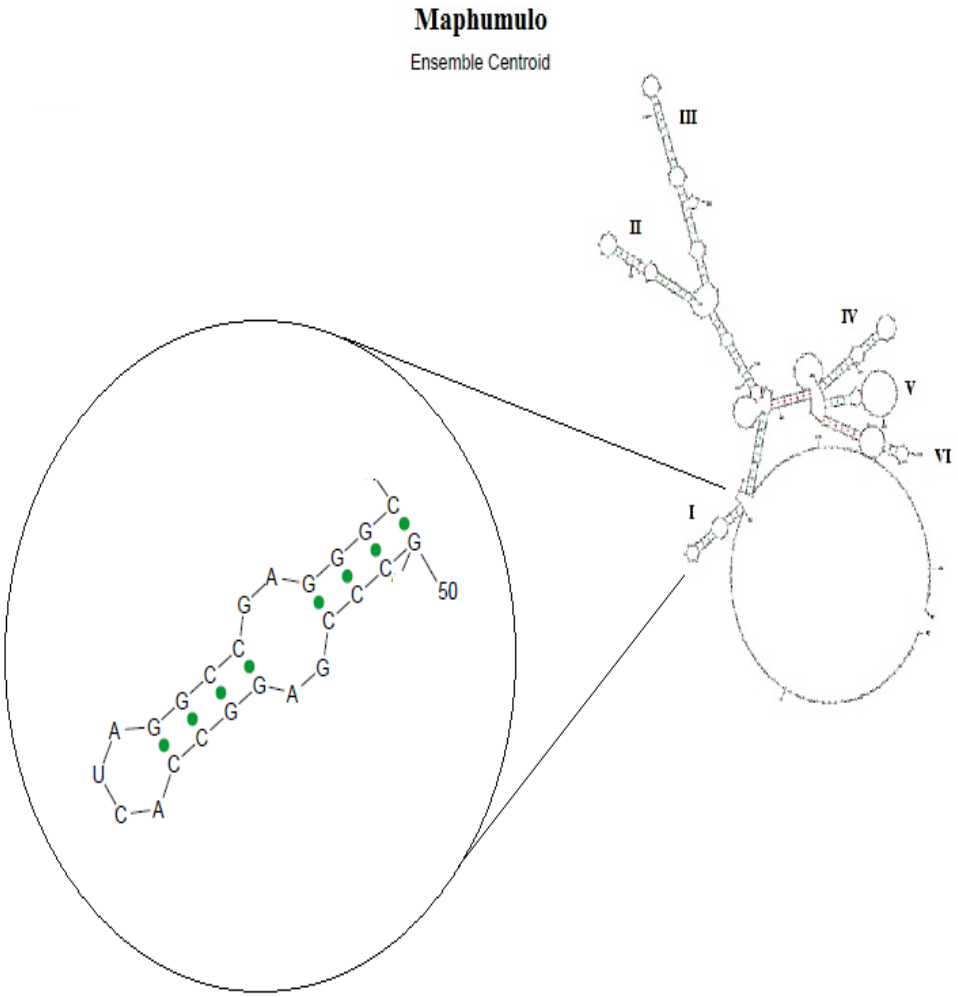

Figure 2. Highlights the common motif in all the accessions: 5'-GCCCGAGGCCACUAGGCCGAGGGC-3' at approximately nucleotide positions (48-73).

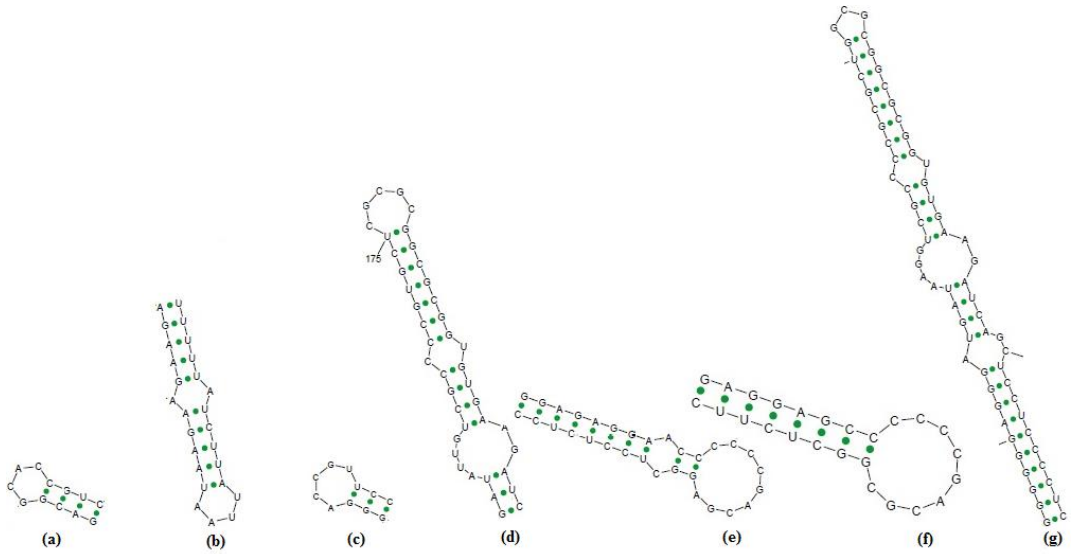

Figure 3. The helices labeled (a) to (g) found in some of the secondary structures of the taro accessions.

Table 2. The conserved motifs (a) to (g) found in taro secondary structures.

(a)	(b)	(c)	(d)	(e)	(f)	(g)
Mangozi	Modderfontein	Willowvale	Ntumeni	Tshwane1	Isipingo	Cocoindia
Makatini	Eshowe2	Isipingo	Lusikisiki	Tshwane2	Hluhluwe	Jozini
MakatiniRes	Lusikisiki	Hluhluwe	Mbazwana	Eshowe1	Eshowe1	
Nigeria	Tshwane1	Eshowe1				
	Mbazwana	Mbazwana				
	Pietermaritzburg	Tshwane1				
		Cocoindia				
		Pietermaritzburg				
		Jozini				

accessions strengthens the potential of the ITS2 region in identifying groups of accessions in taro based on their molecular characteristics. The analysis of compensatory base changes (CBCs) in the ribosomal RNA (ITS2) secondary structure has been used to successfully verify the taxonomy of closely related species. CBCs occur in a paired region of a primary RNA transcript when both nucleotides of a paired site mutate, while the pairing itself is maintained. This is significant in the sense that discrimination between two closely related species by a single CBC can be achieved (Muller et al., 2007). CBCs in the ITS2 secondary structures are found to correlate strongly with distinct biological species. This study was not able to identify a working computer programme for species classification in taro.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Chen AC, Chang CC, Wei NV, Chen CH, Lein YF, Lin HE, Dai CF, Wallace CC (2004). Secondary structure and phylogenetic utility of the ribosomal Internal Transcribed Spacer 2 (ITS2) in scleractinian corals. Zool. Stud 43:759-771.

Chen S, Yao H, Han J, Liu C, Song J, Shi L, Zhu Y, Ma X, Gao T, Pang X, Luo K, Li Y, Li X, Jia X, Lin Y, Leon C. (2010). Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. PloSone 5:1-8.

Dassanayake RS, Gunawardene YINS, Nissanka BDD, Silva KD (2008). ITS-2 secondary structures and phylogeny of *Anopheles culicifacies* species. Bioinform. 2(10):456-460.

Ding Y, Lawrence CE (2003). A statistical sampling algorithm for RNA secondary structure. Nucleic Acids Res. 31(24):7280-7301.

Hershkovitz MA, Zimmer EA (1996). Conservation patterns in angiosperm ITS2 sequences. Nucleic Acids Res. 24(15):2857-2867.

Joseph N, Krauskopf E, Vera MI, Michot B (1999). Ribosomal internal transcribed spacer 2 (ITS2) exhibits a common core of secondary structure in vertebrates and yeast. Nucleic Acids Res. 27(23):4533-4540.

Kreike CM, Van Eck HJ, Lebot V (2004). Genetic diversity of taro [*Colocasia esculenta* (L.) Schott], in Southeast Asia and the Pacific. Theor. Appl. Gen. 109(4):761-768.

Kuracha M, Rayavarapu B, Kumar S, Rao P (2006). Comparison of Secondary Structure of the ribosomal Internal Transcribed Spacer 2 (ITS2) Of eight Lepidopteran species from diverse geographical locations. J. Entomol. 3(3):222-230.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007). Clustal W and Clustal X version 2.0. Bioinformatics 23(21):2947-2948.

Mai JC, Coleman AW (1997). The internal transcribed spacer 2 exhibits a common secondary structure in green algae and flowering plants. J. Mol. Evol. 44(3):258-271.

Muller T, Philippi N, Dandekar T, Schultz J, Wolf M (2007). Distinguishing species. RNA 13(9):1469-1472.

Oliveiro M, Barco A, Modica MV, Richter A, Mariottini P (2009). Ecological bar-coding of coral ivory by second internal transcribed spacer sequences: hosts of coralliophiline gastropods detected by the cnidarian DNA in their stomach. Mol. Ecol.Res. 9(1):94-103.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984). Ribosomal DNA spacer length polymorphism in barely:Mendelian inheritance, chromosomal location and population dynamics. P.N.A.S. (USA) 81:8014-8018.

Saitou N, Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4(4):406-425.

Salvi D, Bellavia G, Cervelli M, Mariottini P (2010). The analysis of rRNA sequence-structure in phylogenetics. An application to the family Pectinidae (Mollusca: Bivalvia). Mol. Phylogenet. Evol. 56(3):1059-1067.

Schultz J, Maisel S, Gerlach D, Muller T, Wolf M (2005). A common core of secondary structure of the internal transcribed spacer 2 (ITS2) throughout the Eukaryota. RNA 11(4):361-364.

Sharma K, Mishra AK, Misra RS (2008). The genetic structure of taro: a comparison of RAPD and isozyme markers. Plant Biotechnol. Rep. 2(3):191-198.

Shange LP (2004).Taro [*Colocasia esculenta* (L.) Schott] production by small-scale farmers in KwaZulu-Natal: Farmer practices and performance of propagule types under wetland and dryland conditions. MSc. Thesis. University of KwaZulu-Natal, Pietermaritzburg.

Soltis PS, Soltis D, Doyle J (1992). Molecular systematics of plants, Chapman and Hall, New York. 434: 1-13.

Tamura K, Peterson D, Stecher G, Nei M, Kumar S (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol.Evol. 28(10):2731-2739.

Trimanto T, Sajidan RN, Sugiyarto YS (2011). Characterization of taro (*Colocasia esculenta*) based on morphological and isozymic patterns markers. Nus.Biosci. 2:7-14.

Van Nues RW, Rientjes MJ, Morré SA, Mollee E, Planta RJ, Venema J, Raué HA (1995). Evolutionarily conserved structural elements are critical for processing of Internal Transcribed Spacer 2 from *Saccharomyces cerevisiae* pre-cursor ribosomal RNA. J. Mol. Biol. 250(10):24-36.

Wang JK, Higa S (1983). Taro: a review of *Colocasia esculenta* and its potentials, University of Hawaii Press, Honolulu.
White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols: A guide to Methods and Applications (ed. M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White), Academic Press: San Diego, U.S.A. 18(1):315-322.