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Molecular phylogenetic authentication of the relative evolution of *Desplatsia spp.* from Southern Nigeria

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ABSTRACT: *Desplatsia spp.* Is among threatened species on the list of the International Union for Conservation of Nature (IUCN). On the taxonomic classification of the species, they are reported as belonging to Tiliaceae by some authorities while others report as Malvaceae family. In this study, Random Amplified Polymorphic DNA markers (RAPD) was used to evaluate the genetic relatedness of *Desplatsia subericarpa* and *Desplatsia dewevrei* collected from Southern Nigeria with the aim of ascertaining their exact plant family. Fresh leaves samples of test plants were collected alongside already established plant members of Tiliaceae (*Corchorus olitorius*) and Mavaceae (*Abelmoschus esculentus*). The genomic DNA was extracted from leaves using Bioline isolate II plant Genomic DNA kit. Three-cluster analysis was conducted based on Nei's genetic distance matrices. Results showed clear RAPD bonding patterns. The combination of 10 random primers generated 84 bands all of which were polymorphic (100%). Findings suggest that though closely related to the family Tiliaceae, both species of Desplatsia are neither relatives of Tiliaceae nor Malvaceae families. Further and advanced study is recommended to appropriately classify *Desplatsia spp*. into a plant family to avoid the disparity in their present taxonomic classification.

Keywords: *Desplatsia spp.*; Southern-Nigeria; Phylogeny; Tiliaceae; Malvaceae; RAPD

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Introduction

Random Amplified Polymorphic DNA (RAPD) analysis is a multi-locus arbitrary finger-printing technique useful in determining genetic relationships of various species (1-3). RAPD analyses are efficient, economical and tend to produce genetic markers suited to the assessment of population, race and species-specific genetic variation (4). Genetic variations between plant materials may result from variations in DNA sequences and ecological effects. "The assessment and maintenance of genetic variation, which involves the use of biochemical and molecular markers, is crucial for providing a fount of adaptability to environmental stress (5)". Several efficient genetic markers are used to reveal genetic variability within and among the same set of plant samples, including random amplified polymorphic DNA (RAPD)-based polymerase chain reaction (PCR), a DNA marker, and isozymes, protein markers. These markers differ from each other with respect to genomic abundance, level of polymorphism detected, locus specificity, reproducibility, technical requirements, cost, and the type of data generated (6, 7). RAPD has been used for the assessment of genetic relationships and variation in *Paspalum vaginatum* (8), variation in populations of *Ranunculus reptans* (9) and *Changeful smyrnioides* (10).

RAPD was the first PCR- based molecular markers to be employed in genetic variation analysis (11, 12).

Desplatsia (or *Desplatzia*) is a genus of small trees native to tropical Africa formerly classified as Tiliaceae. It is distributed across West African countries; Ivory Coast, West Cameroons, across the Congo Basin to Rwanda, Ghana and the Southern Nigeria (13, 14). The genus was initiated by Bocquillon in 1867, with a single species (*Desplatsia subericarpa*) though, the genus contains a few more species (15, 16). In addition to *Desplatsia subericarpa*, the other more recognized species are *Desplatsia chrysochlamys*, *Desplatsia lutea* and *Desplatsia dewevrei* with *Desplatsia caudata*, *Desplatsia chrysophylla*, *Desplatsia floribunda*, *Desplatsia klainii*, *Desplatsia mildbraedii* and *Desplatsia trillesiana* as other recorded names (13). According to Burkil (17), *Desplatsia* (*subericarpa*) belongs to the plant family Tiliaceae while *Desplatsia dewevrei* belongs to the family Malvaceae according to Ken Fern (18) and Hassler (14). The experimental question; Is it possible for two species of the same genera to belong to different plant families? It is certain that phenotypic traits can be reliable measures of genetic differences (19). Phenotypic variation is positively associated with genetic diversity, but it is dependent on environmental factors as well as on the interaction between genotypes (20). Morphological characters may be unstable due to environmental influences; so that methods to assess and detect genetic diversity have extended from analysis of discrete morphological traits to biochemical and molecular traits (21). Therefore, morphological characterization which allows analysis of discrete morphological traits to biochemical and molecular traits (21) in the presence of environmental variation (5) is necessary. This research is aimed at defining the genetic relatedness or differences amongst *Desplatsia* spp in order to verify if the *Desplatsia* spp are of the family Tiliaceae or Malvaceae,

Materials and Methods

DNA Extraction:

Fresh leaf samples of *Desplatsia subericarpa*, *Desplatsia dewevrei*, *Corchorus olitorius*, and *Abelmoschus esculentus* were dried and preserved in silica gel until need for DNA extraction. Total genomic DNA was extracted from these leaf samples using Bioline Isolate II Plant Genomic DNA kit according to the manufacturer's protocol. The DNA obtained was quantified using Nanodrop Spectrophotometer and the integrity was verified on 1% agarose gel at African Biosciences Ltd Ibadan, Nigeria.

PCR Amplification and RAPD analysis:

The PCR amplification was performed using Solis Biodyne FirePol Ready-To-Load PCR Master mix and 10 random decamers. A 10 µl reaction was prepared for each sample per primer. Each 10 µl reaction contains; 2 µl master mix (5x), 1 µl primer (10 µM), 2 µl template DNA (10 ng/µl) and 5 µl nuclease-free water. The PCR program includes an initial denaturation at 95^{o}C for 3 min, denaturation at at 94^{o}C for 30 secs, annealing at 37^{o}C for 1 min, extension at 72^{o}C for 30 sec and final extension at 72^{o}C for 10 min. The denaturation, annealing and extension steps were allowed to run for 40 cycles. The fragment analysis was performed on 2% agarose gel in 1x TBE buffer at 80v for 50 mins. The gel was stained precast with ethidium bromide to a concentration of 0.5 µg/ml. The list of primers used in the study and their sequences are presented thus;

Primers	***Sequence (5' → 3')***
OPA 03	AGTCAGCCAC
OPA 13	CAGCACCCAC
OPA 15	TTCCGAACCC
OPA 17	GACCGCTTGT
OPA 19	CAAACGTCGG
OPAB 02	TGATCCCTGG
OPAB 06	TGCTCTGCCC
OPAB 08	GTCCACACGG
OPAB 11	GTAGACCCGT
OPAB 14	TCCGCTCTGG

Data Analysis:
The gel bands were scored into a binary matrix using Pyelp 1.4 (22). The estimation of Nei's genetic distances and construction of the phylogenetic tree (UPGMA algorithm) were on Genalex 6.502 (23) and MEGA 7 (24) respectively.

Results and Discussion

Genomic DNA isolated from fresh leaves of *Desplatsia* spp; *Desplatsia subericarpa* and *Desplatsia dewevrei* alongside *Abelmoschus esculentus* and *Corchorus olitorius* which served as outgroups were investigated in this study. Figure 1 shows the gel images from the 10 primers used. Presence of a band was scored as 1 and its absence as 0. The binary matrix was manually edited and missing values were represented as -1.

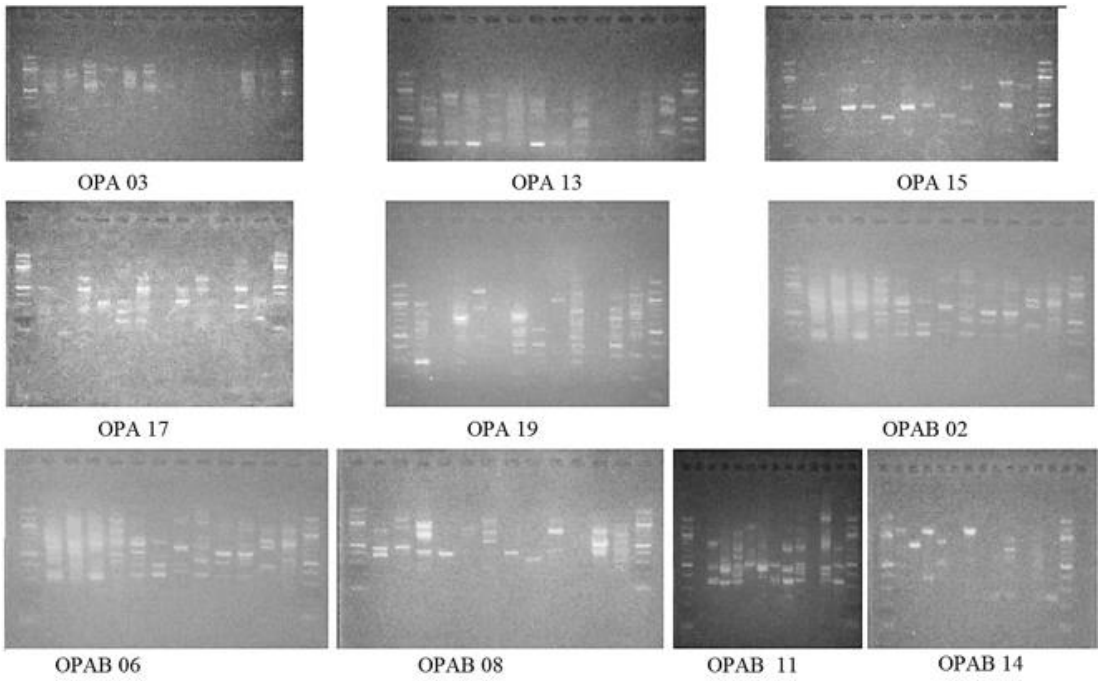

Figure 1: RAPD Fingerprints for the 10 primers used in this study.

Key: Order of samples on gel images: M 1 2 3 4 5 6 7 8 9 10 11 12 M
M- DNA Ladder (100bp)
Desplatsia subericarpa: 1 2 3 4 7 8
Desplatsia dewevrei: 3 5 6 9 10
Abelmoschus esculentus: 11
Corchorus olitorius: 12

The combination of the 10 random primers generated 84 bands, all of which were polymorphic (100%). The 84 polymorphic bands contained 20 unique and 64 non-unique bands (Table 1). *Desplatsia subericarpa* and *Desplatsia dewevrei* had 58 detectable bands out of which bands 10 and 12 were private bands unique to each species. *Abelmoschus esculentus* had 30 while *Corchorus olitorius* had 23 detectable bands out of which 2 and 3 were private (Figure 2).

Table 1: Bands distribution and polymorphism revealed by the primers

Primers	Total No of Bands	Monomorphic (Common) Bands	Polymorphic Bands		Percentage Polymorphism
			Unique	Non-unique	
OPA 03	4	0	0	4	100
OPA 13	11	0	0	11	100
OPA 15	6	0	3	3	100
OPA 17	11	0	4	7	100
OPA 19	12	0	4	8	100
OPAB 02	5	0	3	2	100
OPAB 06	9	0	1	8	100
OPAB 08	9	0	2	7	100
OPAB 11	10	0	2	8	100
OPAB 14	7	0	1	6	100
Total	**84**	**0**	**20**	**64**	**100**

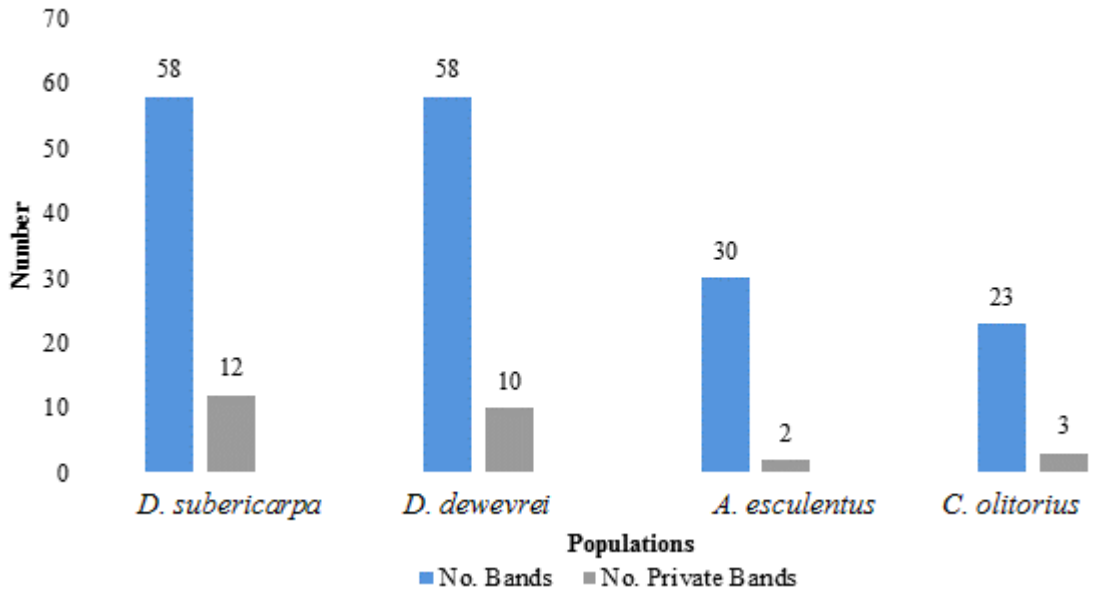

Figure 2: Band patterns across populations

The Nei's genetic distance matrix revealed that *A. esculentus* and *C. olitorius* are genetically farther apart from each other than each is from the *Desplatsia spp* (Table 2). This portrays a triangular relationship which can be easily visualised in the Principal Coordinate Analysis PCoA (Figure 3). The Principal Coordinate Analysis (PCoA) based on Nei's genetic distances with data standardization showed that the first, second and third coordinates accounted for 63.24, 34.21 and 2.55% of the observed variations respectively (Table 3). The triangular relationship demonstrates that the genus *Desplatsia* is closer to *A. esculentus* for some genetic characters, but closer to *C. olitorius* for other genetic characters. For characters captured by the first coordinate (which covers most of the variations observed), the *Desplatsia spp* are closer to *C. olitorius* but closer to *A. esculentus* on the fewer variations captured by the second coordinate (Table 3 and Figure 6).

Table 2: Nei's pairwise genetic distance matrix

	D. subericarpa	*D. dewevrei*	*A. esculentus*	*C. olitorius*
Desplatsia subericarpa	0.000			
Desplatsia dewevrei	0.096	0.000		
Abelmoschus esculentus	0.354	0.367	0.000	
Corchorus olitorius	0.293	0.311	0.461	0.000

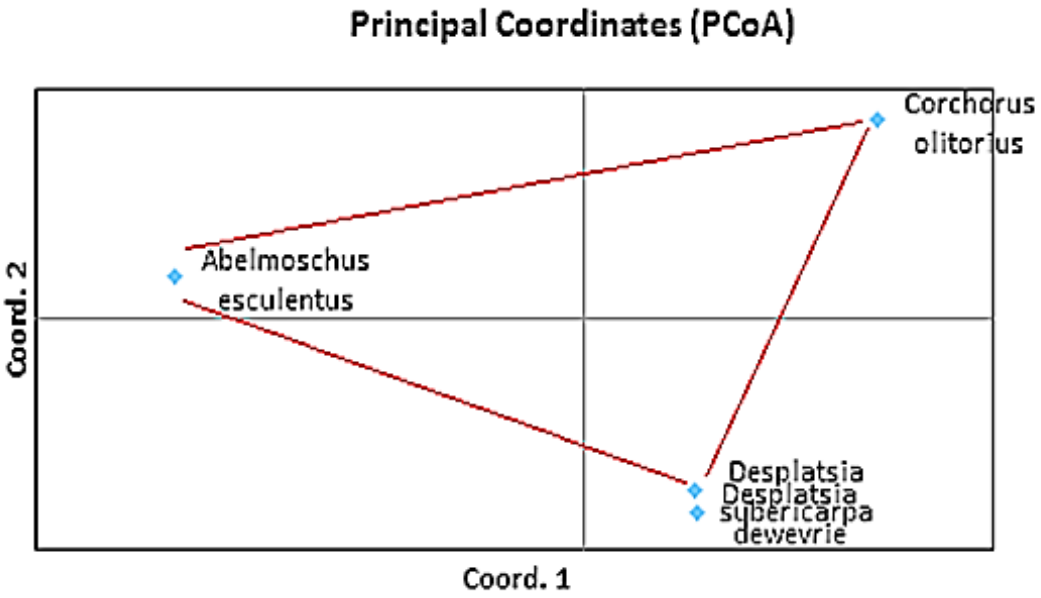

Figure 3: Principal Coordinate Analysis (PCoA) of *A. esculentus*, *C. olitorius* and *Desplatsia spp.*

The phylogenetic tree in Figure 4 revealed 3 main clusters (A, B and C), one of which belongs to the *Abelmoschus esculentus* and *Corchorus olitorius* (Cluster C). The *Desplatsia* spp formed two close and chimeric clusters. Figure 5 is a condensed phylogenetic tree constructed from the Nei's genetic distance (Table 3) between the different populations.

Table 3: Eigen values and proportions by axis and sample Eigen vectors

Axis No.	1	2	3
%	63.24	34.21	2.55
EigenValue	0.037	0.020	0.002
Desplatsia subericarpa	0.041	-0.074	-0.029
Desplatsia dewevrei	0.042	-0.084	0.026
Abelmoschus esculentus	-0.150	0.019	0.000
Corchorus olitorius	0.108	0.086	0.001

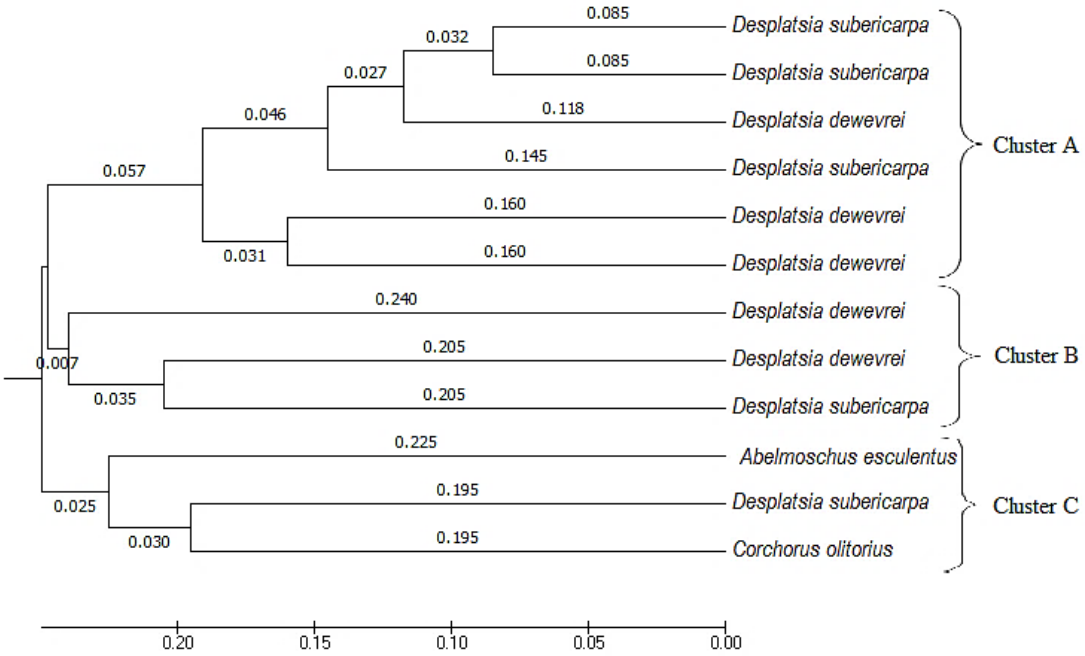

Figure 4: Sample phylogenetic tree – Unweigted Paired Group Method with Averages (UPGMA)

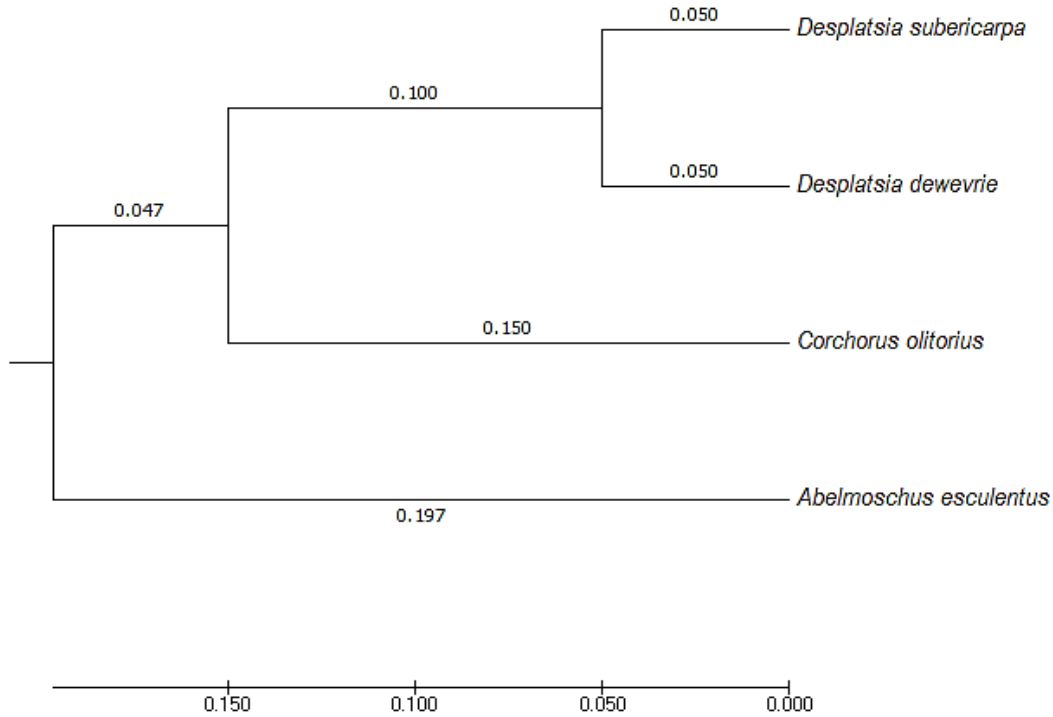

Figure 5: Population phylogenetic tree (UPGMA)

The population phylogenetic tree revealed a closer relationship between *Desplatsia* spp and *Corchorus olitorius* than between the former and *Abelmoschus esculentus.* Though the core families that make up Malvales (Bombaceae, Malvaceae, Sterculiaceae and Tiliaceae) form a well-supported monophyletic group within Malvales, the only one of the four core families that represent a monophyletic group is Malvaceae (15, 25). This may, therefore, suggests why *Desplatsia* spp share some genetic similarities with the family Tiliaceae and Malvaceae. However, *Desplatsia* spp did not closely cluster with any of the two type species and in fact, the cluster pattern observed from the

phylogenetic trees and PCoA in this study suggests that *Desplatsia subericarpa* and *Desplatsia dewevrei* belong to an entirely different family that is neither Tiliaceae nor Malvaceae. Perhaps they belong to Grewioideae as suggested by hinsley (13) and seconded in studies carried out by Brunken and Muellner (26). Further/ advance research involving more samples of the suspected plant family members (Tiliaceae, Malvaceae, Grewioideae) is recommended.

Conclusion

The present study is the first report on the genetic diversity of *Desplatsia* spp using RAPD method. It is recommended that more studies including other related and well-established family type species be carried out with the aim of verifying if *Desplatsia* spp truly belongs to other families than Malvaceae and Tiliaceae.

References

1. Ramshini H, Naghavi MR, Alizadeh H. Comparison of genetic diversity based on total and sharp bands of RAPD data in wheat. Asian J. Plant Sci. 2005; 4(2):123-127.
2. Sadder MT, Ateyyeh AF. Molecular assessment of polymorphism among local Jordanian genotypes of the common fig (*Ficus carica* L.). Sci. Hort. 2006; 107: 347-351.
3. Shinde VM, Dhaliwal K, Mahadik KR, Joshi KS, Patwardhan BK. RAPD analysis for determination of components in herbal medicine. Evid. Based Complement Alternat. Med. 2007; 4: 21-23.
4. Aagaard JE, Krutoviskii KV, Strauss SH. RAPDs and allozymes exhibit similar levels of diversity and differentiation among population and races of Douglas fir. Heredity, 1998; 81: 69-78.
5. Mondini L, Noorani A, Pagnotta MA. Assessing plant genetic diversity by molecular tools. Diver. 2009; 1: 19-35.
6. Goncaves MB, Suetterlin P, Yip P, et al. Neurogenesis in an age-dependent manner. Mol. Cell. Neurosci. 2008; 38: 526-536.
7. Kumar RV, Tripathi YK, Shukla P, Ahlawat S. Genetic diversity and relationships among germplasm of *Jatropha curcas* L. revealed by RAPDs. Trees- Struct. Funct. 2009; 23: 1075- 1079.
8. Lin ZW, Jarret RL, Duncan RR et al. Genetic relationships and variation among ecotype of seashore paspalum (*Paspalum vaginatum*) determined by random amplified polymorphic DNA markers. Genome 1994; 37: 1011–1017.
9. Fischer M, Husi R, Daniel P, et al RAPD variation among and within small and large populations of the rare clonal plant *Rananculus reptans* (Rananculaceae). Am. J. Bot. 2000; 87(8): 1128- 1137.
10. Fu C, Qiu Y, Kong H. RAPD analysis of genetic diversity in *Chagium smyrnioides* (Apiaceae), an endangered plant. Bot. Bull. Acad. Sin. 2003; 44: 13-18.
11. Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acid Res. 1990; 18: 7213-7218.
12. Schierwater B, Ender A. Different thermostable DNA polymerase may apply to different RAPD products. Nucleic Acids Res. 1993; 21: 4647-4648.
13. Hinsley SR. Classification of Malvaceae: Overview, composition; position; division, Malvaceae Info (Home), (2006). Accessed on 20 December 2019, @ http://www.malvaceae.info/Classification/overview.html.
14. Hassler M. World Plants: Synonymic Checklists of the Vascular Plants of the World. In: Species 2000 & ITIS Catalogue of Life, 2016 Annual Checklist (Roskov Y., Abucay L., Orrell T., Nicolson D., Flann C., Bailly N., Kirk P., Bourgoin T., DeWalt R.E., Decock W., De Wever A., eds). Digital resource at www.catalogueoflife.org/annual-checklist/2016. Species 2000: Naturalis, Leiden, the Netherlands.
15. Kubitzki K, Chase MW. Introduction to Malvales. *In:* Kubitzki, K., Bayer, C. (Eds.). The Families and Genera of Vascular Plants, vol. V, Flowering Plants, Dicotyledons: Expanded Caryophyllales, Capparales and Malvales. Springer, Berlin, 2003; 12–16.
16. Hutchinson J. The Genera of Flowering Plants (Angiospermae).Clarendon Press, Oxford. 1967; 2: 536-567.
17. Burkill HM. The useful plants of West Tropical Africa.2nd Edition.Volume 1, Families A–D. Royal Botanic Gardens, Kew, United Kingdom. 1985.
18. Ken F. Useful Tropical Plants Database. Available online @ <tropical.theferns.info/viewtropical.php?id=Desplatsia+subericarpa>. 2014
19. Singh SK, Singh CM, Lal GM. Assessment of genetic variability for yield and its component characters in rice (*Oryza sativa* L.). Res. Plant Biol. 2011; 1: 73-76.
20. Moose SP, Mumm RH. Molecular plant breeding as the foundation for 21st-century crop improvement. Plant Physiol. 2008; 147: 969- 977.

21. Muthusamy S, Kanagarajan S, Ponnusamy S. Efficiency of RAPD and ISSR markers system in accessing genetic variation of rice bean (*Vigna umbellata*) landraces. Electronic J. Biotech. 2008; 11(3): 1-9.
22. Pavel AB, Vasile CL. PyElph- A Software Tool for Gel Images and Pylogenetics. BMC Bioinfo. 2012; 13(9).
23. Peakall R, Smouse PE. GENALEX 6: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research – An Update. Bioinfo. 2006; 28: 2537- 2539.
24. Kumar S, Stecher G, Koichiro T. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0. Mol. Biol. Evol. 2015.
25. Nyfeller R, Bayer C, Alverson WS, et al. Phylogenetic analysis of the *Malvadendrina clade* (Malvaceae s.I.) based on plastid DNA sequence. Org. Div. Evol. 2005; 5: 109-123
26. Brunken U, Muellner AN. A New Tribal Classification of Grewioideae (Malvaceae) Based on Morphological and Molecular Phylogenetic Evidence. Syst. Bot. 2012; 37 (3): 699-711.