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Molecular Characterization of *Basella alba* L. and *Basella rubra* L. using Random Amplified Polymorphic DNA Profiling

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Abstract
The limitation of morphological characterization in revealing genetic variation in species has been the predisposition of most quantitative characters and some qualitative characters to environmental influences. This has necessitated the use of molecular methods in the characterization of species in the recent time. This study was designed to examine and characterize *Basella alba* and *Basella rubra* using Random Amplified Polymorphic DNAs (RAPDs) profiling in order to provide useful information that could enhance the elucidation of their taxonomic status. Genomic DNA of fresh leaf samples of the two species were isolated and subsequently subjected to RAPD analysis by scoring for presence (1) or absence (0) of bands. Monomorphic and polymorphic bands were identified, number of amplified alleles determined, percentage polymorphism established, genetic diversity and genetic distance were calculated. The study revealed 123 individual fragments obtained from the 11 primers used, with 112 (91.06%) of the fragments being monomorphic, with 11 being polymorphic. The genetic variation between the species studied was 8.94%, while the genetic distance was 0.21. The results obtained indicated that the *Basella alba* and *Basella rubra* studied were quite close genetically, thus suggesting that there could be a possibility of gene flow between them.

Keywords: Polymorphic, Monomorphic, Genetic distance, Genetic variation, Genomic screening

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Introduction

Basella alba L. and *Basella rubra* L. are considered as underutilized vegetables that belong to the family Basellaceae. They are commonly known as Ceylon spinach and locally called "Amunututu" in Southwestern Nigeria. They are commonly grown for their young shoots, which make a succulent, slightly mucilaginous vegetable (Oloyede et al*.,* 2013). They have several ethnobotanical uses which include treatment of gonorrhoea, constipation, leprosy, dysentery, ulcer and burns (Dixit and Goyal, 2011), intestinal disorders (Singh et al*.,* 2010), earache and sore throat (Chatchawal et al*.,* 2010; Paul et al*.,* 2011).

The use of morphological characters in revealing genetic variation in species is often limited since the expression of some of the characters, especially the quantitative traits, are usually strongly influenced by environmental factors (Oladejo et al., 2019; Bolaji, 2020). DNA-based techniques have been utilized in recent times as complementary strategies to traditional approaches for the assessment of genetic diversity in species (Alam et al., 2012; Gayathree et al., 2020; Fayaz et al., 2022; Korattukudy et al., 2022). Random amplified polymorphic DNA (RAPD), a PCR based technique, has been used by researchers (Kumar and Gurusubramanian, 2011; Alam et al., 2012; Bolaji, 2020; Sobowale et al., 2020) in the identification of genetic variation in plant species.

Basella rubra and *Basella alba* are considered as two distinct species on the basis of leaf

characters and stem colour (Cook, 2010). However, their taxonomic status has since generated a lot of controversies as some researchers treat the two names as synonymous (Warrier et al*.,* 1996); some treat them as separate species (Larkcom, 2007; Oladele and Aborishade, 2009); while others treat them as varieties (Roy et al*.,* 2010). The specific objective of this research is therefore to characterize *Basella alba* and *B. rubra* using RAPD profiling in order to provide additional information that can assist in elucidating their taxonomic status.

Materials and Methods

Plant Materials Studied and Source

Seeds of *Basella alba* and *Basella rubra* were obtained from the National Centre for Genetic Resource and Biotechnology (NAGRAB), Nigeria, National Horticultural Research Institute (NIHORT), Nigeria and Obafemi Awolowo University, Ile-Ife, Nigeria. The seeds were sown in soil contained in polythene bags and raised in the screenhouse of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The plant samples were authenticated at the IFE Herbarium of the Department of Botany, Obafemi Awolowo University, Ile Ife, Nigeria. Fresh leaves harvested from the plant samples were used for the molecular analysis in this study.

Molecular Analysis

Random Amplified Polymorphic DNA profiling of the plant samples studied was carried out at the National Horticultural Research Institute (NIHORT), Nigeria following the method of Williams et al. (1990) with some modifications as explained in the methods below.

Isolation of genomic DNA

The total genomic DNA was isolated from fresh leaf samples using the SDS method (Dellaporta et al., 1983; Oladejo et al., 2019; Bolaji 2020; Teniola et al., 2021). Lysing of the fresh leaf samples was carried out mechanically after 1000 µL of extraction buffer was added to 0.75 g of each of the samples using mortar and pestle. The mixture was transferred into a sterile Eppendorf tube placed in water bath at 65^{o}C for 30 min and then it was allowed to cool for 5 min. A 200 µL of 5 M potassium acetate was then added.

The Eppendorf tube was inverted and gently placed on ice for 20 min after which 600 µL of CIA (chloroform isoamyl alcohol) was added. The vortex mixture was spun at 5000 rpm for 10 min in a centrifuge. Thereafter, the supernatant was decanted into another sterile Eppendorf tube. A 500 µL of cold isopropanol was added and the mixture was gently mixed for 2-3 min, after which, it was placed in - 80^{o}C freezer for 30 min to enhance DNA precipitation.

The mixture was centrifuged at 4000 rpm for 20 min and the supernatant was removed before adding 400 µL of 70% ethanol. It was then flapped to ensure floating of DNA pellet. It was centrifuged again at 3500 rpm for 10 min and the supernatant decanted. This was repeated twice and then the pellet was air-dried at room temperature to ensure that no trace of ethanol was left. A 100 µL of sterile distilled water and 5 µL of RNase were then added. The mixture was spun for 3 seconds and then placed in an incubator set at 37^{o}C for 30 minutes. This was stored at -20^{o}C until needed for polymerase chain reaction (PCR) analysis.

RAPD-PCR reactions

Genomic screening was conducted with 11 random decamer primers (Table 1). The polymerase chain reaction (PCR) was performed in a total reaction volume of 10 µL containing (Table 2). Amplification of DNA was carried out in a thermocycler using the thermal profile as detailed in (Table 3). After completion of the cycle, PCR products were stored at -20^{o}C until required for electrophoresis.

The products of amplification were loaded on 1.5% (w/v) agarose gel with 0.5× TBE buffer. Electrophoresis was carried out for 90 min at 100 V, running simultaneously with 10 µL of 100 bp loaded on the gel as molecular standard. Visualization, taking of photographs and analysing of the gel followed.

RAPD data analysis

The RAPD data analysis included scoring for the presence or absence of bands, identification of monomorphic and polymorphic bands, determination of number of fragments amplified and calculation of percentage polymorphism. The Percentage polymorphism of the primers used was calculated as Percentage polymorphism = No of polymorphic bands/Total number of bands multiplied by 100. The genetic variation (PD%) between *Basella alba* and *Basella rubra* was calculated as PD = $[F_{ab}/(F_a+F_b)] \times 100$; where F_{ab} = number of fragments that differ between two individuals 'a' and 'b'; F_a = number of

fragments scored in DNA profile of 'a'; F_b = number of fragments scored in DNA profile of 'b' (Gilbert et al., 1991).

Calculation of genetic distance between *Basella alba* and *Basella rubra* was carried out using the modified Rogers Distance (GD_{MR}) formula calculated as $GD_{MR}= [(N_{10} + N_{01})/2N]^{0.5}$; where N_{10} = number of bands (alleles) present only in an individual '*i*'; N_{01}= numbers of bands present only in an individual '*j*'; N = total number of bands (Mohammadi and Prasanna, 2003).

Table 1: Arbitrary RAPD primers used in this study

S/N	Name of Primer	Primer Sequence
1	OPH-02	- 5′ – TCG GAC GTG A – 3′
2	OPT-08	- 5′ – AAC GGC GAC A – 3′
3	OPT-05	- 5′ – GGG TTT GGC A – 3′
4	OPT-07	- 5′ – GGC AGG CTG T – 3′
5	OPT-20	- 5′ – GAC CAA TGC C – 3′
6	OPT-01	- 5′ – GGG CCA CTC A – 3′
7	OPH-07	- 5′ – CTG CAT CGT G – 3′
8	OPH-01	- 5′ – GGT CGG ACA A – 3′
9	OPH-05	- 5′ – AGT CGT CCC C – 3′
10	OPB-08	-5′ – GTG CAC ACG G – 3′
11	OPB-05	- 5′ – TGC GCC CTT C – 3′

Table 2: PCR mixture used in the study

Reagent	Volume (µL)
10× PCR buffer	1.0
50 mM $MgCl_2$	0.4
5 pMol Forward Primer	0.5
5 pMol Reverse Primer	0.5
DMSO	0.8
2.5 mM DNTPs	0.8
5u/µL Taq Polymerase	0.1
100 ng/µL DNA	3.0
H_2O (Nuclease-free)	2.9
Total	10.0

Table 3: PCR program used in the study

		40 Cycles			
Initial denaturation	Denaturation	Annealing Temperature	Extension	Final Extension	Holding Temperature
94°C	94°C	37°C	72°C	72°C	10°C
5 min	30 sec	30 sec	1 min	7 min	∞

Results

The genomic DNA isolated showed clear bands on 1.5% agarose gel (Plate 1). All the 11 primers employed in this study produced RAPD fragments with bands varying in number and intensity (Plate 2). Primer OPH-02 produced the lowest number of fragments with a total number of four bands, while primer OPB-08 and OPB-05 produced the highest number of fragments with sixteen bands each. Primer OPT-01 gave the highest percentage polymorphism (42.86%) while primers OPH-02, OPT-05, OPH-01 and OPB-05 did not produce any polymorphic band.

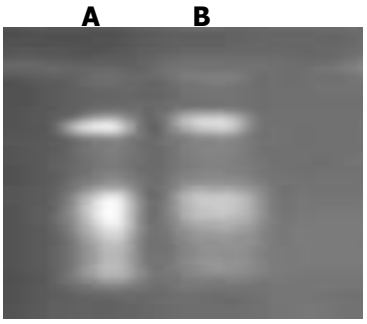

Plate 1: Gel Representation of DNA Extracted from the *Basella* species Studied.
Legend: A = represents DNA from *Basella alba*; B = represents DNA from *Basella rubra*

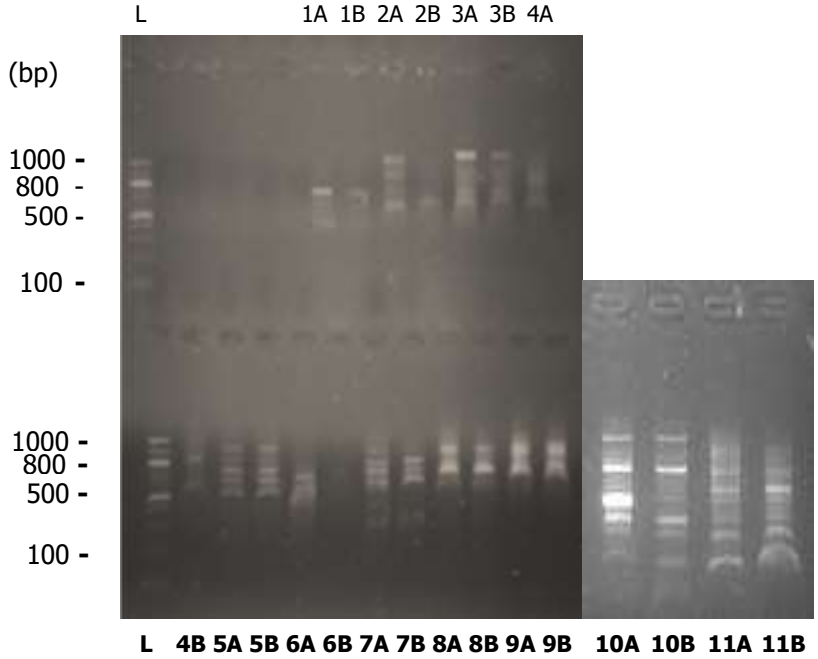

Plate 2: Gel Representation of RAPD Banding Patterns of the *Basella* Species Studied

Legend: L – 100 bp ladder; A - *Basella rubra*; B - *Basella alba*; 1 to 11- primers H02, T-08, OPT-05, OPT-07, OPT-20, OPT-01, OPH-07, OPH-01, OPH-05, OPB-08, OPB-05 respectively

Altogether, 123 individual fragments were obtained from the 11 primers used, with 112 being monomorphic and 11 being polymorphic (Table 4). The genetic variation between *Basella alba* and *Basella rubra* samples used in this study is 8.94%; while the genetic distance is 0.21.

Table 4: Total bands scored by the primers and their percentage polymorphism

Primers	Total number of alleles	Monomorphic bands	Polymorphic bands	Percentage polymorphism
OPH02	4	4	0	0
OPT08	11	10	1	9.09
OPT05	10	10	0	0
OPT07	11	10	1	9.09
OPT20	13	12	1	7.86
OPT01	7	4	3	42.86
OPH07	13	12	1	7.69
OPH01	12	12	0	0
OPH05	10	8	2	20
OPB08	16	14	2	12.05
OPB05	16	16	0	0
	Total = 123	Total = 112 (91.06%)	Total = 11 (8.94%)	

Discussion

According to Alam et al. (2012), DNA amplification products depend on the sequence of the random primers and their compatibility within the genomic DNA. Upadhyay et al. (2004) also noted that the number of markers detected by each primer depends on the primer sequence and the extent of genetic variation, which is genotype specific.

Primer OPT-01 that gave the highest percentage of polymorphism could be further utilized in the study of genetic diversity analysis within the species studied. The polymorphic RAPD marker bands produced could as well be isolated and developed into locus-specific co-dominant markers. According to Kumar and Gurusubramanian (2011) as well as Bolaji (2020) polymorphic RAPD marker bands could be isolated, amplified, cloned and sequenced to produce locus-specific co-dominant markers such as the Sequenced Characterized Amplified Region (SCAR) marker.

The dominance of monomorphic fragments in the RAPD data obtained indicates that the two *Basella* species are quite similar genetically. The relatively small genetic variation (8.94%) as well as genetic distance (0.21) obtained further revealed that they are quite similar genetically. These findings imply that since they are quite close genetically, there could be a possibility of gene flow between them.

Based on their morphological characterization, some researchers (Warrier et al*.,* 1996) were actually of the opinion that the two *Basella* forms were not separate species but synonyms, while others including Henry et al*.* (1987) and Roy et al. (2010) referred to them as varieties. The findings in this study further corroborate the fact that *Basella alba* and *Basella rubra* are not only morphologically similar but are genetically close; the main difference being the purple colouration of *Basella rubra* and the green colouration of *B. alba*. It is however highly recommended that further studies be carried out to establish the possibility of crossability between *B. alba* and *B.rubra* so as to ascertain if there are reproductive isolating mechanisms that could prevent them from hybridizing to produce viable and fertile offspring generally expected of members of the same species.

Conclusion

It was revealed from this study that *Basella alba* and *Basella rubra* are quite close genetically with respect to their DNA profiling, genetic variation, and genetic distance, thus implying that there could be possibility of gene flow between them.

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