Asian Journal of Biology

10(4): 1-8, 2020; Article no.AJOB.61727
ISSN: 2456-7124

Assessment of Genetic Diversity in Indian Spinach (*Basella spp* L) Accessions Using RAPD Markers

Olalekan Ibrahim Sobowale[1,2*], Benjamin Oluwole Akinyele[2], Alexander Chukwunweike Odiyi[2], Adeyela Ibironke Okunlola[2], Emmanuel Oluwakayode Ajayi[3] and Omolara Loveth Aderonmu[3]

[1]Crop Improvement Division, Cocoa Research Institute of Nigeria, Ibadan, Nigeria.
[2]Department of Crop, Soil and Pest Management, Federal University of Technology Akure, Ondo State, Nigeria.
[3]National Horticultural Research Institute, P.M.B. 5432, Idi-Ishin, Jericho, Ibadan, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author OIS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BOA, ACO, AIO, EOA and OLA managed the analyses of the study. Author OIS managed the literature searches. All authors read and approved the final manuscript

Article Information

DOI: 10.9734/AJOB/2020/v10i430111
Editor(s):
(1) Dr. Jehad M. H. Ighbareyeh, Al-Quds Open University, Palestine.
Reviewers:
(1) Leandro Jorge Riverón Cruzata Las Tunas, University of Medical Sciences, Cuba.
(2) Zerihun Jida Buta, Wolkite University, Ethiopa.
Complete Peer review History: http://www.sdiarticle4.com/review-history/61727

Original Research Article

Received 22 July 2020
Accepted 28 September 2020
Published 21 November 2020

ABSTRACT

Background: Indian spinach is one of the important underexploited tropical leafy vegetables which have high nutritional and medicinal value. Molecular marker technology has greatly accelerated the process involved in breeding programs for the improvement of various crops and its techniques have been considered to be the most suitable means of estimating genetic diversity.
Aim: The study is to determine the genetic diversity among the accessions of Indian spinach collected from South western Nigeria using molecular markers.
Methodology: Random amplified polymorphic DNA (RAPD) markers were used to assay 20 accessions of Indian spinach (*Basella spp*) collected from the south western states of Nigeria (Oyo, Osun, Ogun, Ondo and Ekiti).
Results: Results showed that RAPD markers were highly polymorphic and generated alleles ranging from two to eight. The polymorphic information content was highest for the OPT-17 primer

**Corresponding author: E-mail: sobolekky@hotmail.com;*

(0.757) and the mean average was (4.23) Moreover, gene diversity (0.785) was high, and cluster analysis delineated the accessions into five groups, which indicated that a significant genetic diversity was present among the accessions studied. A dendrogram clustering method revealed five major clusters. Clusters I, II and IV had one accessions each, III had four and V had thirteen accessions.

Conclusion: The result revealed that RAPD markers are useful for genetic characterization as they provide information on the interspecific and phylogenetic statuses of the accessions. The markers also showed a genetic variability that could be exploited for varietal delineation and improvement of the vegetable in Nigeria.

Keywords: Indian spinach; genetic diversity; RAPD – Random Amplified Polymorphic DNA.

1. INTRODUCTION

Indian spinach is one of the important underutilized tropical leafy vegetables widely adapted to a variety of soils and climates [1]. It is a fast growing plant which belongs to the family *Basellaceae*. Indian spinach plays an important role in nutrition, medicine, ornamental and industrial use. It is an important part of human diet as it is found to possesses calcium, iron, magnesium, vitamin A, vitamin C and vitamin B9 (folic acid) and several vital anti-oxidant [2,3,4]. It is a leafy vegetable often used for the treatment of malaria, leprosy, dysentery and hypertension [5,6,7]. Indian spinach is said to cure burns, sore throat, liver diseases, ulcer, constipation and gonorrhoea [8,9]. Extract from the fruit of Indian spinach has been used for making dye, ink, cosmestic and official seals [10,11,12]. Indian spinach is fed to livestock to increase milk production in some parts of East Africa [13,14]. Red forms of Indian spinach are commonly planted as ornamentals and as pot plants in Europe and North America [14].

Assessment of levels and patterns of genetic diversity among cultivated plants is a fundamental component of crop improvement programs. Apart from the use of morphological markers in the characterization of crop species, molecular markers are equally available which are less time consuming and more reliable than the use of morphological traits. Molecular marker techniques have been considered to be the most suitable means of estimating genetic diversity. RAPD markers have been used to determine and distinguish genetic variation in crops species and among the molecular markers RAPD has been adopted widely for the indentification of genetic relationship among vegetables species. Genetic diversity in *Vernonia amygdalina Delile* accessions was revealed by random amplified polymorphic DNAs (RAPDs) [15]. A study using RAPDs was also conducted by [16] to study the genetic diversity in three grain amaranths. [17] claimed that RAPD analysis clearly revealed genetic diversity among and within accessions of the amaranth species studied, showing that this DNA marker is a useful tool not only for assessing intra specific variation, but also for subsequent characterization and identification of quantitative trait loci (QTLs) for agronomic and nutritional quality in amaranth genotypes. Owing to the nutritional and medicinal importance of Indian spinach, assessment of the extent of genetic variation among its species is essential not only for understanding its pattern of diversity but also for its improvement. This study was, therefore, conducted with the objective of determining the genetic variability and molecular diversity in Indian spinach species in south western Nigeria by using random amplified polymorphic DNA (RAPD) markers.

2. MATERIALS AND METHODS

2.1 Plant Materials and Sample Collection

The seeds of the 20 accessions of Indian spinach collected from the five south western states of Nigeria (Ekiti, Ogun, Ondo, Osun, and Oyo) (Table 1) were raised in soil filled plastic pots in a screen house at the National Horticultural Research Institute (NIHORT) Ibadan, Oyo state, South west, Nigeria (Rain-forest zone) ($3°56^{1}$E and $7°33^{1}$N 168 m above sea level). Fresh young leaves samples of the 20 accessions were harvested at four weeks after planting in well labelled and tightly covered sample bags. The samples were placed on ice pack and immediately conveyed to Bioscience laboratory of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria for analyses.

2.2 DNA Extraction

Approximately 200 mg of plant tissue was ground using liquid nitrogen and transferred into 1.5 ml eppendorf tube after which 700 ul of pre-heated plant extraction buffer was added. The tube was

incubated at 65°C for 20 min and the content mixed by occasionally inverting the tube to homogenize the sample. The tubes were removed and allowed to cool for 2 mins, 500ul of ice-cold 5M Potassium acetate added, incubated on ice for 20 mins to precipitate protein, centrifuged at 12000 rpm for 10 min and the supernatant transferred into freshly labeled tubes. 700 ul of chloroform Isoamylalcohol (24:1) was added and mixed gently to further precipitate protein and lipids. After centrifuging at 12000 rpm for 10 mins, the supernatant was transferred into a new tube and 500 ul of ice-cold isopropanol added, mixed gently, incubated at -80°C for 15 mins to precipitate the DNA and then centrifuged at 12000 rpm for 10 mins. The supernatant was decanted until the last drop. 100 ul of 70% ethanol was added to wash the DNA pellet. The pellet was spun down and air dried until no trace of ethanol was found. 60ul of ultra-pure water was added to re-suspend the DNA. 2 ul of RNase was added and incubated at 37°C for 30-40 mins.

Table 1. List of the accessions of Indian Spinach used in the study

S/N	Accessions	Collection site	State
1	ADE01	Ado	Ekiti state
2	ADE02	Ado	Ekiti state
3	ADE03	Ado	Ekiti state
4	AKR02	Akure	Ondo state
5	IBD01	Ibadan	Oyo state
6	IBD02	Ibadan	Oyo state
7	IBD03	Ibadan	Oyo state
8	IBDLS02	Life seeds	Oyo state
9	IBDLS03	Life seeds	Oyo state
10	IBDLS04	Life seeds	Oyo state
11	IJB	Ijebu	Ogun state
12	IKL02	Ikole	Ekiti state
13	IKL03	Ikole	Ekiti state
14	IKR	Ikire	Osun state
15	ILF	Ile ife	Osun sate
16	OSG01	Osogbo	Osun state
17	OSG02	Osogbo	Osun state
18	OSG04	Osogbo	Osun state
19	OSG05	Osogbo	Osun state
20	OSG06	Osogbo	Osun state

2.3 Cocktail Mix

The PCR cocktail mix consists of 2.5 ul of 10x PCR buffer,1 ul of 25 mM $MgCl_2$, 1 ul of primer, 1 ul of DMSO, 2 ul of 2.5 mM DNTPs, 0.1 ul of Taq DNA polymerase, and 3 ul of 10 ng/ul DNA. The total reaction volume was made up to 25 ul using 14.4 ul Nuclease free water.

2.4 PCR Conditions

Initial denaturation at 94°C for 5 mins, followed by 40 cycles of denaturation at 94°C for 30 sec, annealing at 37°C for 30 secs and elongation at 72°C for 1 min, followed by a final elongation step at 72°C for 7 mins and temperature maintained at 10°C forever. Amplified fragments were visualized on ethidium bromide stained with 1.5% agarose electrophoresis gels.

2.5 Data Acquisition and Analysis

The RAPD alleles were scored in binary codes; clearly visible RAPD bands were assigned 1 for presence, 0 for absence and m for missing data. Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands [18]. The Polymorphic Information Content (PIC), a measure of variability for each locus was calculated across the assay of units by the formula of [19]:

$$PIC_i = 2f_i(1 - f_i)$$

Where f_i is the frequency of the amplified allele (band present), and $(1 - f_i)$ is the frequency of the null allele (band absent) of marker i.

To estimate the level of genetic diversity, genetic similarities were evaluated using Nei and Li/Dice similarity index [20] with the aid of the NTSYSpc software, version 2.11 [21]. A dendrogram was generated from the similarity matix using the UPGMA (unweighted pair group method using arithmetic averages) in NTSYSpc program.

3. RESULTS

Extracted DNA obtained across 20 Indian spinach accessions showed sharp and clear bands. DNA bands did not indicate smearing (degraded DNA) as seen from the agarose gel electrophoresis picture (Plate 1) and the genomic DNA was a satisfactory PCR template. The DNA quantity in nanogram per microliter (ng/µl) of all Indian spinach accessions were well above 100ng/µl (Table 3) which is the maximum quantity of DNA required for a RAPD reaction. The quality of genomic DNA as indicated by the 260/280 ratio was at optimum level for all accessions. The DNA concentrations ranged from 115.6 ng/µl to 956 ng/µl (Table 3).

Table 2. List of Indian spinach RAPD markers used showing the nucleotide sequence

Markers	Sequence (5^1 –3^1)			
OPT – 01	- 5^1 – GGG	CCA	CTC	A – 3^1
OPT – 02	- 5^1 – GGA	GAG	ACT	C – 3^1
OPH – 04	- 5^1 – GGA	AGT	CGC	C – 3^1
OPT – 05	- 5^1 – GGG	TTT	GGC	A – 3^1
OPT – 06	- 5^1 – CAA	GGG	CAG	A – 3^1
OPT – 10	- 5^1 – CCT	TCG	GAA	G – 3^1
OPT – 12	- 5^1 – GCG	TGT	CTA	G – 3^1
OPT – 16	- 5^1 – GGT	GAA	CGC	T – 3^1
OPT – 17	- 5^1 – CCA	ACG	TCG	T – 3^1
OPB – 04	- 5^1 – GGA	CTG	GAG	T – 3^1
OPB – 08	- 5^1 – GTG	CAC	ACG	G – 3^1
OPB – 11	- 5^1 – GTA	GAC	CCG	T – 3^1

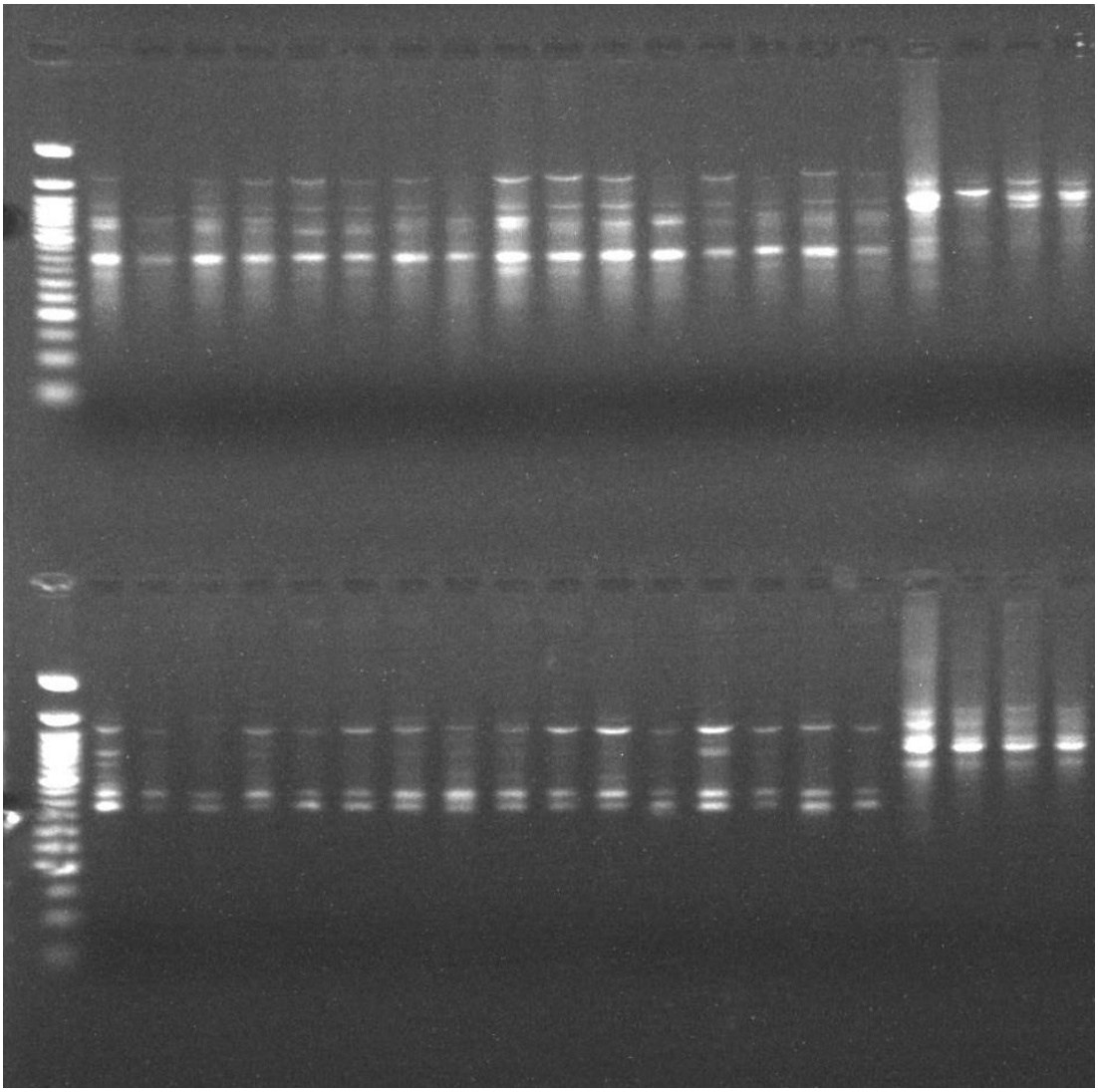

Plate 1. DNA of 20 accessions of Indian spinach visualized on Agarose gel electrophoresis

Table 3. Spectrophotometer (nanodrop) values of twenty accessions of Indian spinach

ID no	Accessions	ng/µl	260/280
1	ADE01	528.84	1
2	ADE02	449.1	1.39
3	ADE03	662.99	1.35
4	AKR02	775.08	1.05
5	IBD01	411.2	1.63
6	IBD02	229.49	1.33
7	IBD03	173.1	1.73
8	IBDLS02	231.4	2.01
9	IBDLS03	246.4	0.91
10	IBDLS04	115.6	1.6
11	IJB	398.65	2.56
12	IKL02	123.7	2.33
13	IKL03	394.31	1.61
14	IKR	166.8	1.4
15	ILF	354.61	2.14
16	OSG01	405.3	4.03
17	OSG02	262.2	1.43
18	OSG04	460.2	2.02
19	OSG05	956.4	2.42
20	OSG06	126.3	1.97

The number of alleles, gene diversity and polymorphism information content (PIC) of Indian spinach accessions RAPD markers are presented in Table 4. The 12 RAPD markers exhibited extensive polymorphism among the Indian spinach accessions. However the major allele frequency ranged from 0.350 to 0.900. Genetic diversity of the primer used ranged from 0.095 to 0.785, where OPT17 revealed the highest genetic diversity (Table 4). The allele number per RAPD marker ranged from 2 to 8 with an average of 4 per marker. The RAPD marker OPT17 was the most informative, detecting the highest number of alleles which was 8 among the Indian spinach accessions tested, while OPT02, OPB08, and OPB11 had the least, each having 2 alleles. Markers OPT01, OPT05, and OPT10 had 4 alleles each. PIC for Indian spinach varied from 0.090 (OPT02) to 0.757 (OPT17) with an average of 0.423 (Table 4).

Fig. 1 is a dendrogram showing genetic similarity among the 20 accessions of Indian spinach as revealed by RAPD markers. At similarity level of 0.78, all the accessions formed a single cluster. The dendrogram also showed that the first linkage was formed between IBDLS04 and IBD01 at 1.00 similarity level. At 0.89 similarity level, the dendrogram revealed five distinct groupings. Group I, II and IV had one accession each OSG06, IBDLS02 and ADE03 respectively. Group III was composed of four accessions, namely IBDLS03, OSG05, IKL02 and IBD02. Group V comprised thirteen accessions, namely (AKR02, OSG04, OSG02, IBD03, IKL03, IKR, OSG01, ADE02, ILF, IBDLS04, IBD01, IJB and ADE01) (Fig. 1).

4. DISCUSSION

RAPD technique is increasingly being employed for population studies and it provides valuable data on diversity through its ability to detect variations at the DNA level. In this study, RAPD analyses provided an insight into the genetic diversity, genetic structure and distribution of Indian spinach obtained from south western states of Nigeria. RAPD markers were used to determine the extent of the genetic diversity in different plant species, including bitter leaf plant, *Vernonia amygdalina Delile* [15], Eggplant,

Table 4. Number of alleles, gene diversity and polymorphism information content (PIC) of Indian spinach RAPD markers

Markers	Major allele frequency	No of alleles	Gene diversity	PIC
OPB04	0.350	7	0.690	0.658
OPT02	0.900	2	0.095	0.090
OPT12	0.450	5	0.660	0.611
OPT16	0.650	3	0.405	0.368
OPB08	0.650	2	0.495	0.372
OPB11	0.850	2	0.255	0.222
OPT01	0.800	4	0.410	0.379
OPT05	0.700	4	0.480	0.450
OPT06	0.650	6	0.550	0.526
OPT10	0.800	4	0.270	0.259
OPT17	0.400	8	0.785	0.757
OPH04	0.850	3	0.445	0.381
Mean	0.671	4	0.462	0.423

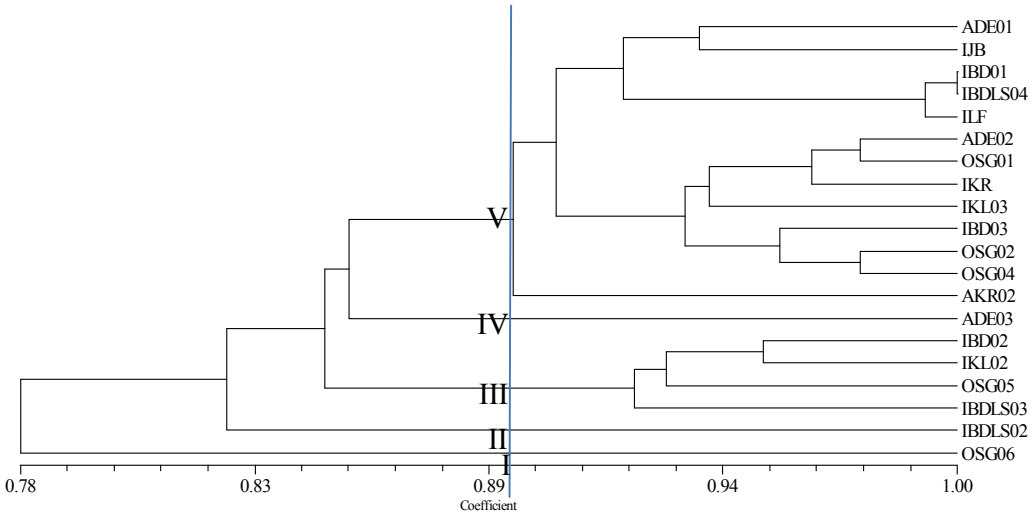

Fig. 1. Molecular Dendrogram showing genetic similarity among the twenty accessions of Indian spinach based on RAPD markers

Solanum melongena [22] and Capsicum [23]. The technique provided useful information for the exploitation of available genetic variability. The number of alleles and the high gene diversity (0.785) observed in this study proved that significant genetic variability occurs among the Indian spinach accessions. [24] reported that the polymorphism information content (PIC) values can be classified into three categories (i) if the PIC value of the marker is more than 0.5, the marker is considered as highly informative, (ii) if the PIC value ranges from 0.25 to 0.5, the marker is moderately informative, and (iii) if the PIC value is less than 0.25, then the marker is slightly informative. The average PIC value of 0.423 obtained in the current study is moderately informative suggesting that the RAPD marker employed in the study was very useful for diversity study in Indian spinach. Twelve of the polymorphic RAPDs primers used in this study were found useful for the delineation of accessions collected from different parts of south western state of the country. These observations are similar to those reported by [22] where eleven primers were used to distinguish 19 genotypes of *Solanum melongena.* Likewise a report by [15] where five primers were used to distinguish 30 accessions of *Vernonia amygdalina*. Also, a report by [25] shows that six primers were used to distinguish all cultivars of the sweet cherry studied. Similarly, [26] successfully used seven RAPD primers to distinguish juniper and cedar cultivars. The five clusters identified in this study showed that the association of the accessions within the clusters was not based on the geographical similarities. Three accessions, namely I (OSG06), II (IBDLS02) and IV (ADE03) failed to form any cluster. Members of each of these three accessions were separated from one another, thereby making them the most diverse accessions. The accessions from the same location fell into different clusters while the same cluster contained accessions from different locations. This implies that clustering pattern of accessions was not influenced by their geographical distribution.

5. CONCLUSIONS

The study showed that a significant genetic diversity exists within the studied Indian spinach representatives. The RAPD technique produced many clusters from the twenty accessions and revealed variation within the clusters. This picture provides a basis for further investigation into the genetic diversity of Indian spinach using other marker systems such as the co-dominant microsatellites (SSRs), single-nucleotide polymorphisms (SNPs) and a sequence analysis to unravel more genetic information and locate important mutations that are responsible for some of the observed phenotypic differences.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Reddy MT, Begum H, Sunil N, Pandravada SR, Sivaraj N, Kumar S. Preliminary characterization and evaluation of landraces of Indian spinach (*Basella* spp. L.) for agro-economic and quality traits. Plant Breed. Biotech. 2014;2(1):48-63.
2. Deshmukh SA, Gaikwad DK. A review of the taxonomy, ethnobotany, phytochemistry and pharmacology of *Basella alba* (Basellaceae). Journalof Applied Pharmaceutical Science. 2014; 4(01):153-165.
3. Bamidele O, Akinnuga, AM, Olorunfemi JO, Odetola OA, Oparaji, CK, Ezeigbo N. Effects of aqueous extract of *Basella alba* leaves on haematological and biochemical parameters in albino rats. African Journal of Biotechnology. 2010;9(41):6952-6955.
4. Palada MC, Chang LC. Suggested cultural practices for Basella. AVRDC International Cooperators Guide; 2003. AVRDC Pub No. 03-553.
5. Kumar P. Indian spinach, *Basella alba* (PUI) succulent, branched, smooth, twining herbaceous vine. Best Nutrition; 2010.
6. Olowokudejo JD, Kadiri AB, Travih VA. An ethnobotanical survey of herbal markets and medicinal plants in Logos State of Nigeria. Ethnobotanical Leaflets. 2008;12: 851-865.
7. Vincent PKT, Denis Z, Moses NN. The antimalarial potential of medicinal plants used for the treatment of malaria in Cameroonian folk medicine. African Journal of Traditional Complementary and Alternative Medicines. 2008;5:302-321.
8. Paul AK, Arif HA, Seraj S, Nahar A, Nasrin D, Chowdhury MH, Islam F, Jahan R, Bashar A, Freedman R, Rahmatullah M. A survey of plant items eaten by the low income groups of the rural population of Talbunia village in Bagerhat district, Bangladesh with an account of their folk medicinal applications. American – Eurasian Journal of Sustainable Agriculture. 2011;5:132-144.
9. Singh PK, Kumar V, Tiwari RK, Sharma A, Rao CV, Singh RH. Medico-Ethnobotany of Chatara block of district Sonebhadara, Uttar Pradesh, India. Advances in Biological Research. 2010;4:65-80.
10. Chatchawal C, Nualkaew N, Preeprame S, Porasuphatana S, Priprame A. Physical and biological properties of mucilage from *Basella alba* L. stem and its gel formulations. International Journal of plant Sciences. 2010;6:104-112.
11. Olgorite A. Genetic relationship between *Basella alba* and *Basella rubra*. WARA Newsletter. 2006;9-10.
12. Odilora CA, Ozabar WC, Ugiagba B. Extraction and coloring effect of the dye from *Basella alba* (Indian spinach) on cotton and polyester fibres. Proceeding of the Annual Conference of the chemical al society of Nigeria held at Calabar; 2002.
13. Rodda R, Kota A, Sindhuri T, Kumar SA, Gnananath K. Investigation on anti-inflammatory property of Basella alba Linn leaf extract. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(1):452-454.
14. Grubben GJ, Denton OA. Plant Resources of Tropical African vegetables. Wageningen; Backhuys Leiden (CTA). Wageningen: PROTA Foundation; 2004.
15. Aikpokpodion PO, Abebe J, Igwu D. Genetic diversity in *Vernonia amygdalina* Delile accessions revealed by random amplified polymorphic DNAs Journal of Biotechnology, Computational Biology and Bionanotechnology. 2018;99(2):147–152.
16. Mandal N, Das PK. Intra- and interspecific genetic diversity in grain Amaranthus using random amplified polymorphic DNA markers. Plant Tissue Culture. 2002;12: 49–56.
17. Akin-Idowu PE, Gbadegesin MA, Orkpeh U, Ibitoye DO, Odunola OA. Characterization of Grain Amaranth (*Amaranthus* spp.) Germplasm in South West Nigeria Using Morphological, Nutritional, and Random Amplified Polymorphic DNA (RAPD) Analysis Resources; 2016.
DOI: 10.3390/resources5010006
Available:www.mdpi.com/journal/resources
18. Martos V, Royo C, Rharrabti Y, Garcia del Moral LF. Using AFLPs to Determine Phylogenetic Relationships and Genetic Erosion in Durum Wheat Cultivars Released in Italy and Spain throughout the 20th Century. Field Crop Res. 2005; 91:107-116.
19. Roldán-Ruiz I, Dendauw J, Van Bockstaele E, Depicker A, De Loose M. AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). Molecular Breeding. 2000;6:125–134.
20. Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of

the National Academic of Science, USA. 1979;76:3269-3273.

21. Rohlf, FJ. NTSYSpc Numerical Taxonomy and Multivariate Analysis System. Exeter Software, Setauket, New York. 1998;2.
22. Demir K, Bakir M, Sarikamis G, Acunalp S. Genetic diversity of eggplant (*Solanum melongena*) germplasm from Turkey assessed by SSR and RAPD markers Genet. Mol. Res. 2010;9(3):1568-1576.
23. Rabelo da Costa F, Perreira TNS, Vitoria AP, DeCampos MG, Rodriges R, DaSilva H, Perreira MG. Genetic diversity among Capsicum accessions using RAPD markers Crop Breed. Appl. Biotech. 2006;6:18–23.
24. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 1980;32:314-331.
25. Lisek A, Korbin M, Rozpara E. Using simply generated RAPD markers to distinguish between sweet cherry (*Prunus avium* L.) cultivars. J. Fruit Ornam. Plant Res. 2005;14:53–59.
26. Hsiang T, Huang J. The use of RAPD markers to distinguish among juniper and cedar cultivars. Can. J. Bot. 2000;78(5): 655–659.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/61727