EVALUATION OF GENETIC DIVERSITY OF SELECTED CASTOR OIL PLANT (*Ricinus communis* L.) ACCESSIONS USING RAPD MOLECULAR MARKERS

***[1]Muhammad, A. M., [2]Falusi, O. A., [2]Daudu, O. A. Y., [3]Salihu, B. Z., [2]Muhammad, L. M. and [2]Haruna, H. B.,**

[1] Department of Biological Sciences, Ibrahim Badamasi Babangida University, Lapai, Nigeria
[2] Department of Biological Sciences, Federal University of Technology, Minna, Nigeria
[3] Castor Genetic Improvement Unit, National Cereals Research Institute (NCRI), Badeggi, Bida, Niger State, Nigeria

ABSTRACT

In order to assess the genetic diversity among the Nigerian castor oil plants accessions (*Ricinus communis* L.) twelve accessions Viz: Acc 006, Acc 012, Acc 016, Acc 019, Acc 026, Acc 027, Acc 038, Acc 040, Acc 042, Acc 043, Acc 045 and Acc 048 were grown to evaluate the level of genetic diversity among the selected castor oil plants. Thus the aim of the study was to assess the level of genetic diversity among castor genotypes using molecular tools. Deoxyribonucleic acid (DNA) was extracted and purified from leaves of the plant using fermentas kit, Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) technique was used to detect the variations in DNA profile. Thirteen RAPDs primers were used, out of which 10 polymorphic primers revealed 100% polymorphism among the castor genotypes, the results showed percentage polymorphism ranges from 76.92% to 100%. OPD-13 had 76.92%, OPA-02, OPA-03, OPA-13, OPB-08, OPD-02, OPD-07, OPD-08, OPE-07 and SIGMA-D-P had 100% respectively. Dendrogram revealed two main clusters that consists all the accessions grouped together in their respective sub-cluster. The first large group was further divided into two sub-groups containing acc 026 and the second sub-group contains Acc 006 and Acc 016. The second large group divided into two sub-groups containing all other accessions. This research revealed high genetic diversity of castor that can be used in future breeding program in Nigeria for increased demand of castor oil for industrial uses.

Keywords: Genetic diversity, Improvement, Dendrogram, RAPD

***Corresponding author:** maaliaisha@gmail.com, +2348069558816.

INTRODUCTION

Castor oil plant (*Ricinus communis*, 2n=20) is a species of flowering plant in the spurge (Euphorbiaceae) family which contains numerous number of plants species including, physic nut (*Jatropha curcas*), rubber tree (*Hevea brasiliensis*) and cassava (*Manihot esculenta*). Castor belongs to a monotypic genus *Ricinus* and sub-tribe *Ricininae* (Weiss, 2000). Castor's origin is obscured due to its wide dissemination in ancient times, and the

ease and rapidity of its establishment as a native plant. However, it is mostly agreed to be a native to Africa, especially Ethiopia (Anjani, 2012).

The crop is now widely revived as a viable industrial crop for all tropical and subtropical regions, addressing the need for commercial crops with low input costs and viable returns (Salihu *et al.*, 2013). Historical summaries of world castor production have been published by Weiss (2000). In 2009 India, China, Brazil and Mozambique produced the majority of the world's castor oil; with Ethiopia, Thailand and Paraguay contributing relatively minor amount (Foster *et al*, 2013)). The annual total world production was 1.5million tonnes (MT), produced on a total area of 1.47million hectare (Mh) (Foster *et al*, 2013)). The average seed yields never exceeded 1,200 kg/ha during this periods (Foster *et al*, 2013).

In 2013, the major world producers of castor seeds were India, China, Mozambique, Ethiopia, Brazil, Paraqwa and South Africa with a total contribution of 1,805,153MT to world production. India produced the largest, about 1,644,000MT, among the countries (FAO, 2013). In Nigeria, hectarage was estimated at about 6000 ha, and production at about 3000 – 4000 MT in 2004. The major producing states then were Cross River and Ebonyi (RMRDC, 2009). Presently, the average yield among the farmers in the country is considerably low, ranging between 200kg - 400kg/ Ha (NCRI, 2013).

Castor is a hardy crop which survives in a wide range of ecology. Basically castor grows throughout the warm temperate and tropical regions, it flourishes under varieties of climatic conditions that its range cannot easily be defined (Weiss, 2000). It grows almost anywhere land is available. Castor is basically a long-day plant, but is adaptable with fewer yields to a wide range of photoperiod. Basically castor requires the same amount of nutrients as other low-demand field crops (Gana *et al.*, 2013).

The use of molecular markers has become a common practice in studies of population structure, genetic diversity for pre-breeding and breeding germplasm and in distinguishing one individual genotype to preserve the property of breeding rights (Abou El-Nasr *et al.*, 2014).

According to Gepts, (2004) Development of new cultivars with traits of interest and good adaptation to specific microclimates is only possible when there is available knowledge about the genetic diversity of the species. Genetic diversity analyses of castor bean germplasm collections worldwide have showed low levels of variability and lack of geographically structured genetic populations, regardless of a marker system used (Foster *et al.*, 2013; Qiu *et al.*, 2010; Allan *et al.*, 2008). The remarkable phenotypic variations observed in castor bean do not seem to reflect a high genetic diversity (Yi *et al.*, 2010).

Unlike other important oilseed crops, as oil palm (*Elaeis guineensis*), soybean (*Glycine max*), sunflower (*Helianthus annuus*), and some Euphorbiaceae species, such as cassava and rubber tree, castor bean diversity is still poorly characterized by means of molecular marker systems (Feng *et al.*, 2009; Sayama *et al.*, 2011; Talia *et al.*, 2010). In fact, the species had been overlooked until the late 2000s, when analyses regarding genetic diversity of

germplasm collections were first published (Allan *et al.*, 2008).

Lakhani *et al.*, (2015) observe that, assessment of genetic variation using molecular markers is crucial for the efficient management and biodiversity conservation of plant genetic resources in gene banks. A large number of polymorphic markers are required to determine genetic relationships and genetic diversity in a reliable manner. This limits the use of morphological and biochemical characters, which are limited in number or lack ample diversity in castor. Further, these analyses have intrinsic disadvantages such as limited numbers of markers, and are often less efficient due to their variation and sensitivity to short-term environmental fluctuations. DNA based molecular analysis tools are ideal for germplasm characterization and phylogenetic studies. RAPD has proven to be helpful in detecting genetic variations and evaluation of genetic diversity and also for identifying germplasm in a number of plant species (Gwanama *et al.*, 2000; Kapteyn and Simon, 2002). The utility of PCR-based marker variations as phylogenetic markers for investigating evolutionary relationships among plants has been clearly established. These techniques are independent of environmental factors and offer significant advantage for species identification in that they are rapid, relatively cheap and eliminate the need to grow plants up to maturity (Lakhani *et al.*, 2015).

MATERIALS AND METHODS

Twelve castor accessions (*Ricinus communis* L.) including small and large seeded types were collected from National Cereals Research Institute Badeggi Bida, Nigeria, to study the genetic diversity at molecular level by RAPD markers. The seeds of each accession were sown and nurtured for four (4) weeks. The young leaves of 30-35 days old were collected in the evening and placed on ice in a cooler and additional ice added on top to preserve the samples for transport to the laboratory where DNA extraction followed.

DNA Extraction

The DNA extraction and RAPD (Random Amplified Polymorphic DNA) was carried out at Biosolution Technologies Centre in Akure, Ondo State, Nigeria. Genomic DNA was extracted from the leaves part of the plant (35 days old) of the twelve (12) accessions (35 days old) of castor and purified using a modified protocol described by Warwick and Gugel (2003) with minor modifications as described by Pham *et al.* (2009). The leaves were washed, freeze-dried to form pellets and grinded using a mortar and pestle. 0.5 gram of the powder was taken from which the genomic DNA was isolated using Fermentas Kit following the manufacturer's instructions. DNA quality and concentration was measured using Nanodrop ND-1000 spectrophotometer.

PCR Cycle Condition

Initial denaturation at 94°C for 2 minutes, denaturation at 94°C for 15 - 30 seconds, Annealing at 50°C for 15 - 30 seconds, Extension at 72°C for 1 minute, final extension at 72°C for 5minutes and hold at 4°C indefinitely.

Data Analysis

The numbers of polymorphic and monomorphic bands (for genetic variability) were determined from the presence or absence of bands. The clearly visible bands in at least one genotype were scored (1) for present and (0) for absent, and the data entered into a data matrix. Percentage polymorphism was calculated as the proportion of polymorphic band over the total number of bands. A polymorphic index (PIC) was calculated as PIC = $1\text{-}p^2\ q^2$ where p is the frequency of bands present and q is frequency of bands absent (Lakhani *et al.*, 2015) Coefficient of similarity trees were produced by clustering the similarity data with the un-weighted pair group method using the Statistical Package for Social Sciences (SPSS) version 20. The similarity coefficient was used to create a dendrogram in order to depict phylogenetic relationship.

RESULT AND DISCUSSION

Banding Patterns and Polymorphism

The result of gel electrophoresis showed different patterns of polymorphic bands obtained from the PCR amplification of the 12 accessions to be 12 bands, and the lowest was 9 polymorphic bands. Thirteen RAPD primers showed polymorphism, out of the thirteen primers used, producing a total of 147 bands, all of which were polymorphic representing 100% polymorphism with exceptions of primers OPD-13, OPF-14 and SIGMA-D-14 which had 76.92%, 92.31% and 93.31% respectively though were also polymorphic (Table 1). The Polymorphic Information Content (PIC) values varied between 0.142 (SIGMA-D-01 and others) and 0.498 (OPA-02) with a total of 3.593 (Table 1).

The Phylogenetic Relationship

The phylogenetic analysis represented in the dendrogram generated two main clusters that consists all the accessions grouped together in their respective sub-cluster (Figure 2). The first large group was further divided into two sub-groups containing solitary accession 026 with related distance of 29.3. The second sub-group containing acc. 006 (46.5) and acc. 016 (20.1) being clustered together. The second large group was further divided into two sub-groups, first sub-group contains acc. 042 (15.6), acc. 043 (29.3), acc. 045 (7.6) and acc. 027 (0.0), acc. 019 (0.0), and acc. 048 (0.0) clustered together. The second large group also further divided into two sub-groups, the first sub-group contains acc. 038 (21.5), while the second sub-group contains acc. 040 (23.7) and acc. 012 (9.6). The genotypes clustered together in a group are closely related than other genotypes of other groups (Figure 2).

Table 1: Successful primers used their Sequence, number of polymorphic bands formed, the Percentage of Polymorphic Bands and Polymorphic information content (PIC).

Primers	Primer Sequence	Number of Bands	Number of Polymorphic Bands	Percentage of Polymorphic Bands (%)	PIC Value
OPA-02	- 5' - TGC CGA GCT G - 3'	10	10	100.00	0.498
OPA-03	- 5' - AGT CAG CCA C - 3'	11	11	100.00	0.427
OPA-13	- 5' - CAG CAC CCA C - 3'	11	11	100.00	0.261
OPB-08	- 5' - GTC CAC ACG G - 3'	10	10	100.00	0.474
OPD-02	- 5' - GGA CCC AAC C - 3'	11	11	100.00	0.142
OPD-07	- 5' - TTG GCA CGG G - 3'	12	12	100.00	0.263
OPD-08	- 5' - GTG TGC CCC A - 3'	11	11	100.00	0.355
OPD-13	- 5' - GGG GTG ACG A - 3'	13	10	76.92	0.142
OPE-07	- 5' - AGA TGC AGC C - 3'	9	9	100.00	0.142
OPF-14	- 5' - TGC TGC AGG T - 3'	13	12	92.31	0.261
SIGMA-D-01	- 5' - AAA CGC CGC C - 3'	11	11	100.00	0.142
SIGMA-D-14	- 5' - TCT CGC TCC A - 3'	13	12	92.31	0.142
SIGMA-D-P	- 5' - TGG ACC GGT G - 3'	12	12	100.00	0.355
TOTAL		147	142	97.04	3.593

The number of amplified fragment varying with size ranging from 100 to 1000 bp.

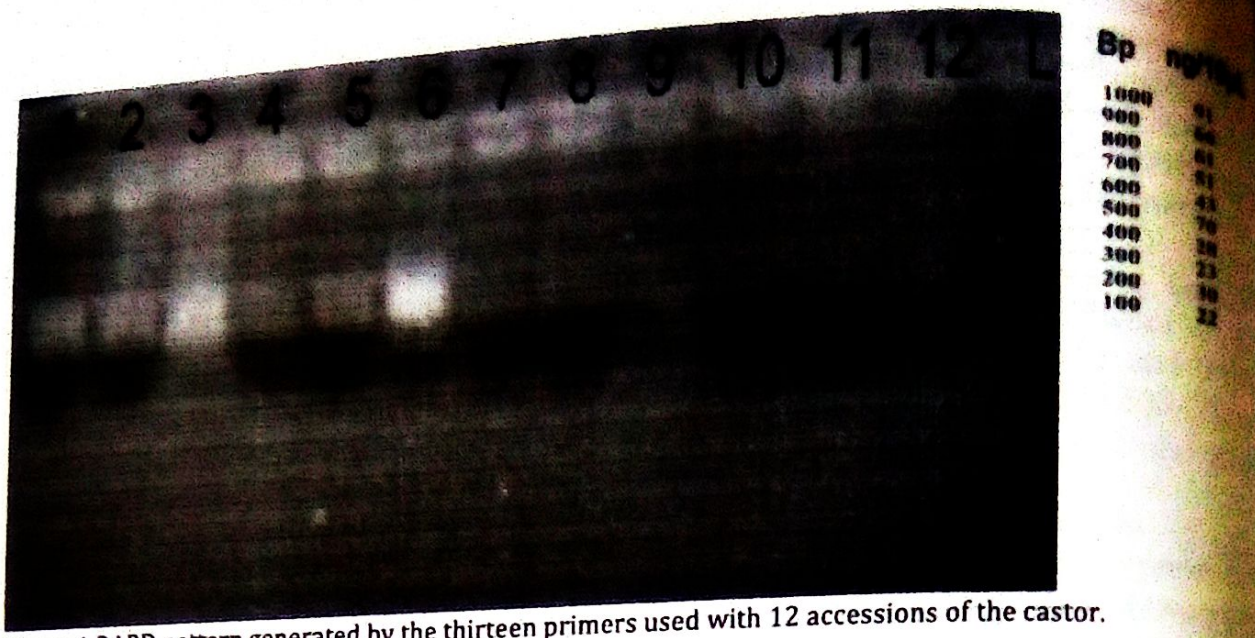

Figure 1 RAPD pattern generated by the thirteen primers used with 12 accessions of the castor.

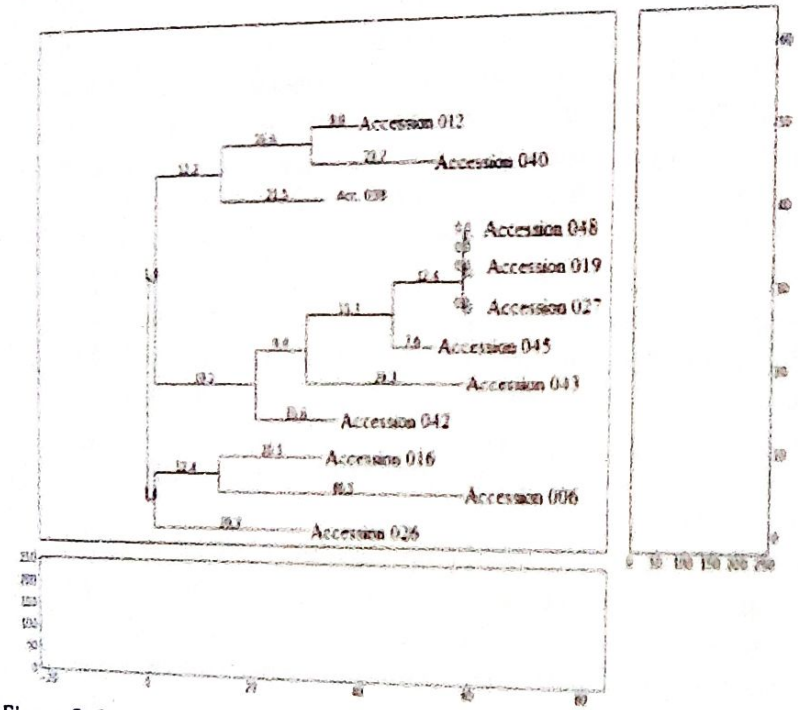

Figure 2: Dendrogram depicting the Genetic relationship among the 12 castor genotypes based on RAPD data.

The percentage polymorphism exhibited by the primers used, producing a total of 147 bands were in close agreement with report of Lakhani, *et al.,* (2015) who also studied genetic diversity in castor using RAPD analysis in India. Similar results have also been reported in castor by Gajera *et al.,* (2010) and Groundnut by Dwivedi *et al.,* (2001).

The phylogenetic relationship observed from the RAPD based dendogram is in close agreement with Lakhani, *et al.* (2015). The unrevealed changes at the molecular level to surface at the morphological level might be due to DNA system of the plant.

In conclusion, this result could aid castor breeding and improvement in Nigeria.

which grouped different accessions into the same clusters.

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