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Phylogenetic and Genomic Relationships Among Melon Populations based on RAPD

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Abstract: Melons belong to the family Cucurbitaceae; it is one of the most important cash crops globally. Their demand is in a very high quantity, especially here in Nigeria. In order to improve the yield of this highly demanded crop, a study was undertaken to ascertain the pattern of its genetic diversity. During this study, eighteen melon cultivars samples grown in south western Nigeria was used; *Cucumis melo*, *Citrullus lanatus* and *Lagenaria siceraria* species. They were investigated using both seed morphology and molecular characters. However, the seed morphological characters revealed, they are sharply pointed with elliptical/oval shape. Analysis of total genomic DNA extracted using CTAB protocol on 1% agarose revealed high molecular weight DNA bands in 88.89% of the samples. While studies on their genetic variation investigated using RAPD analysis, with three primers revealed samples have a wide genetic base with monomorphic bands, which were used to generate UPGMA dendograms. The dendograms showed the examined melons are monophyletic with sample AD001 and AD002 showing distinct morphometric evaluation with 18.00 and 16.94 similarity coefficient, respectively and samples AD003 and AD004 have a closer similarity coefficient at 2.06. Basically, in this study, we have investigated the extent of variation and of relatedness among the 18 melon cultivars, which can now afford breeders the opportunity to make selections on how to improve on the breeding of melon cultivars. However, this research serves as a basis for further characterization of the melon population.

Key words: Melon, RAPD, UPGMA, monomorphic

INTRODUCTION

Melons are a group of members of the plant family Cucurbitaceae commonly called the gourd family, they produce protein and oil rich seeds for which the crop is mainly grown. Members consists species such as *Citrullus lanatus* (Thunb.) Matsum. and Nakai, "Egusi watermelon"; *Cucumeropsis mannii* Naudin "true Egusi" and *Lagenaria siceraria* (Molina) Standl. "bottle gourd" (Achigan-Dako *et al.*, 2008). The Cucurbitaceae or cucurbit family is a large plant family with many useful species found mainly in the tropics. It contains about 119 genera with 825 species which are widely distributed round the world, of which 22 genera and 37 species are indigenous to Nigeria. Fruits of Cucurbitaceae are of great economic importance and one of their main uses apart from their fruits, leaves, flowers and occasionally their root is that of its seeds (Chen *et al.*, 1997; Andres, 2004). Therefore, it is of great importance to study these melon crops because they are economically important and they play a vital role in the well-being of West African rural dwellers as recognized by Andres (2004) in Côte d'Ivoire, Norman (1992) in Achigan-Dako *et al.* (2006) in Benin.

Seeds of cucurbits when freed from the seed coats are can be consumed either raw or cooked, or more usually when ground to a powder to be added to soups and stews (Burkill, 1985). Schippers (2000) and Zoro-Bi *et al.* (2003) summarized the importance of these melon crops for various purposes amidst all, they can serve as weed control plants. They could also be used for human consumption, provision of cash income or other socio-cultural uses as summarized by Achigan-Dako *et al.* (2008). Their seeds are rich in healthy fatty acids, minerals and proteins, which could be ground into paste for preparing local soup or could be fried in vegetable oil to make melon snack "robo". Cucurbits are also used in indigenous medicinal practices; species such as *C. lanatus* subsp. *mucosospermus* is said to heal stomach aches likewise its seed coat in decoction with Eucalyptus (*Eucalyptus camaldulensis*.) roots is a sedative for epilepsy, *Momordica charantia* can be used in treatment of diabetes while roots, stems and leaves of *Coccinia grandis* is used in skin diseases. Fruits are generally eaten as a vegetable in Africa and Asia, the skin of mature fruits are used as containers or in some cases as fishing floats. Leaves are cooked while some species are used for ornamental purposes (Achigan-Dako *et al.*, 2008).

Melons seeds are majorly cultivated for their oil which is of a variable content between 15-45%, consisting of glycerides of linoleic, oleic, palmitic and stearic acid, that could be used for cooking, soap-making; medicinal, vehicle illuminant etc. (Ajibola *et al.*, 1990). The quantity and quality of oil varies between cultivars and there appears to be a field for breeding and selection.

Molecular studies on melon crops revealed they can be manipulated to produce different cultivars that can produce more fruits, seeds and oil. Molecular markers techniques, a powerful tool that have been developed to accurately assess and characterize genetic resources can be employed. These markers are suitable for assessing how much allelic diversity is present in a crop and they have the potential for providing unique fingerprinting for each genetically distinct genotype, a useful means of identifying different cultivars. Likewise, Diversity studies provide useful information for breeders about genetic relationships and distances between individuals. Molecular markers e.g., RAPD, AFLP, ISSR, SSR etc., can exploit the hidden diversity to obtain the benefit from the potential of each species. Therefore, it is a novel tool for classification of cultivars which allows better use of the genetic resources of species. Assessment of genetic diversity among cultivars based only on morpho-agronomic traits might be bias distinct morphotypes can results from few mutations and share a common genetic base, as it has been demonstrated in coca.

Random amplified polymorphic DNA (RAPD) markers have been used by Garcia *et al.* (1998) and Staub *et al.* (2000) to assess the genetic diversity among melon germplasm. Mliki *et al.* (2001) also determined genetic differences among African melon landraces. Katzir *et al.* (1996) have also used Microsatellite markers to evaluate polymorphism in melons. Garco-Rodriguez *et al.* (1996)

using RAPD markers, found that variability among European melon breeds was relatively low compared to lentils and it also revealed low variability.

Breeding for improved product quantity, quality and disease resistance in melon crops cultivars, diversification of fruit and plant types is of considerable commercial interest and their adaptation to specific areas. Since different melon cultivars and species were considered in this study it is of great importance to study their level of genetic diversity which is the aim of this study.

MATERIALS AND METHODS

Plant materials and DNA samples: Eighteen different melon seed cultivars were selected and gotten from south western Nigeria maintained at NACGRAB and others were obtained from different markets in Nigeria (Table 1). The samples were used for DNA extraction following modified Cetyltrimethyl-ammonium Bromide (CTAB) protocol (Doyle and Doyle, 1987). The different melon seeds cultivars were examined qualitatively for seed characters.

PCR amplification: RAPD reactions were assembled and mixed to create a cocktail of 12.5 µL total volume per unit. The cocktail mix contained 10X buffer (1.25 µL), MgCl₂ (1 µL), 5% Tween 20 (1 µL), dNTPs (0.5 µL), *Taq* polymerase (0.2 µL), distilled water (5.55 µL), primer (0.5 µL) and template DNA (2.5 µL). The sample was mixed thoroughly and centrifuged briefly to bring down the contents of the tube. They were placed in a thermocycler for DNA amplification. The PCR was carried out with the RAPD profile of 30 cycles, preheated 105°C, initial denaturing 94°C for 4 min and final extension of 72°C for 6 min.

Table 1: Background of melon seeds used in this study

Botanical names	Location	Date of collection	Collector
<i>Citrullus lanatus</i>	NACGRAB	July, 2011	Oshingboye, A.D.
<i>Citrullus lanatus</i>	NACGRAB	July, 2011	Oshingboye, A.D.
<i>Colocynthis citrullus</i>	NACGRAB	July, 2011	Oshingboye, A.D.
<i>Citrullus lanatus</i>	NACGRAB	July, 2011	Oshingboye, A.D.
<i>Cucumis melo</i>	NACGRAB	July, 2011	Oshingboye, A.D.
<i>Citrullus lanatus</i>	NACGRAB	July, 2011	Oshingboye, A.D.
<i>Citrullus vulgaris</i>	NACGRAB	July, 2011	Oshingboye, A.D.
<i>Lagenaria siceraria</i>	NACGRAB	July, 2011	Oshingboye, A.D.
<i>Cucumis melo</i>	NACGRAB	July, 2011	Oshingboye, A.D.
<i>Citrullus lanatus</i>	NACGRAB	July, 2011	Oshingboye, A.D.
<i>Citrullus lanatus</i>	Mushin market	August, 2011	Oshingboye, A.D.
<i>Citrullus vulgaris</i>	Bariga market	August, 2011	Oshingboye, A.D.
<i>Citrullus lanatus</i>	Bariga market	August, 2011	Oshingboye, A.D.
<i>Citrullus lanatus</i>	Oyingbo market	August, 2011	Oshingboye, A.D.
<i>Citrullus lanatus</i>	Oyingbo market	August, 2011	Oshingboye, A.D.
<i>Citrullus vulgaris</i>	Bariga market	August, 2011	Oshingboye, A.D.
<i>Citrullus vulgaris</i>	Agege market	July, 2011	Oshingboye, A.D.
<i>Cucumis melo</i>	Agege market	July, 2011	Oshingboye, A.D.

Table 2: Morphological characters of melon seeds

Species	Seed size	Seed shape	Seed shape at hilum-end	Seed colour coat
<i>Citrullus lanatus</i>	Large	Elliptical/oval	Sharply pointed	Tan
<i>Citrullus lanatus</i>	Large	Elliptical/oval	Sharply pointed	Yellow
<i>Colocynthis citrullus</i>	Intermediate	Elliptical/oval	Sharply pointed	Yellow
<i>Citrullus lanatus</i>	Intermediate	Elliptical/oval	Sharply pointed	Yellow
<i>Cucumis melo</i>	Large	Elliptical/oval	Sharply pointed	Tan
<i>Citrullus lanatus</i>	Large	Elliptical/oval	Sharply pointed	Brown
<i>Citrullus vulgaris</i>	Intermediate	Elliptical/oval	Sharply pointed	Cream - yellow
<i>Lagenaria siceraria</i>	Large	Elliptical	Sharply pointed	Tan
<i>Cucumis melo</i>	Large	Elliptical	Sharply pointed	Tan
<i>Citrullus lanatus</i>	Intermediate	Elliptical/oval	Sharply pointed	Yellow
<i>Citrullus lanatus</i>	Intermediate	Elliptical/oval	Sharply pointed	Yellow
<i>Citrullus vulgaris</i>	Intermediate	Elliptical/oval	Sharply pointed	-
<i>Citrullus lanatus</i>	Small	Elliptical/oval	Sharply pointed	-
<i>Citrullus lanatus</i>	Very large	Elliptical/oval	Sharply pointed	-
<i>Citrullus lanatus</i>	Intermediate	Elliptical/oval	Sharply pointed	Yellow
<i>Citrullus vulgaris</i>	Intermediate	Elliptical/oval	Sharply pointed	-
<i>Citrullus vulgaris</i>	Intermediate	Elliptical/oval	Sharply pointed	Tan
<i>Cucumis melo</i>	Small	Elliptical/oval	Sharply pointed	Yellow white

Gel scoring and data analysis: Fragments that were clearly resolved on gels were scored as 1 or 0 for present and absent, respectively while bands that could not be confidently scored were regarded as missing. Pair wise distance (similarity) matrices were computed using Sequential Hierarchical and Nested (SAHN) clustering option of the numerical taxonomy and multivariate analysis system NTSYS-pc version 2.02j software package (Rohlf, 1993). The program generated dendograms, which group the tested lines on the basis of Nei genetic distance (Nei and Li, 1979) using unweighted pair group method with arithmetic mean average (UPMGA) cluster analysis (Sneath and Sokal, 1973).

RESULTS

Morphological studies: Examination and description of the qualitative characters of the seeds revealed that they are all sharply pointed and elliptical/oval in shape; *Cucumis melo* has seed ranging from large, intermediate to small size. *Lagenaria siceraria* has large sized seed that is elliptical while *Citrullus lanatus* has seed varying from small, intermediate to large. Descriptive features and terminologies used followed IPGRI 2003 (Table 2).

Molecular evaluation: Polymorphic unambiguous bands that were clearly resolved on gels were scored present (1) or absent (0) while monomorphic bands were also noted. A total of 17 oligonucleotide primers were screened of which 6 produced monomorphic bands while the rest did not amplify DNA at all. The capacity to produce RAPD fragments vary with primers and the species (Mihalte *et al.*, 2011). The UPGMA dendograms generated classified the tested samples into various clusters and sub-clusters at different level of similarity coefficient (Fig. 1). The melon formed a monophyletic group and

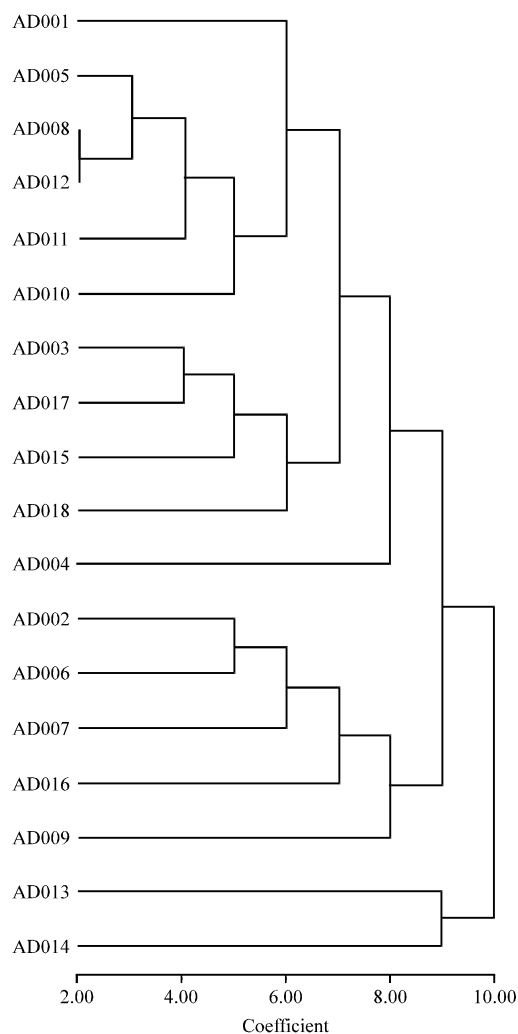


Fig. 1: A UPGMA Dendrogram of 18 melon cultivars based on DNA data analysis

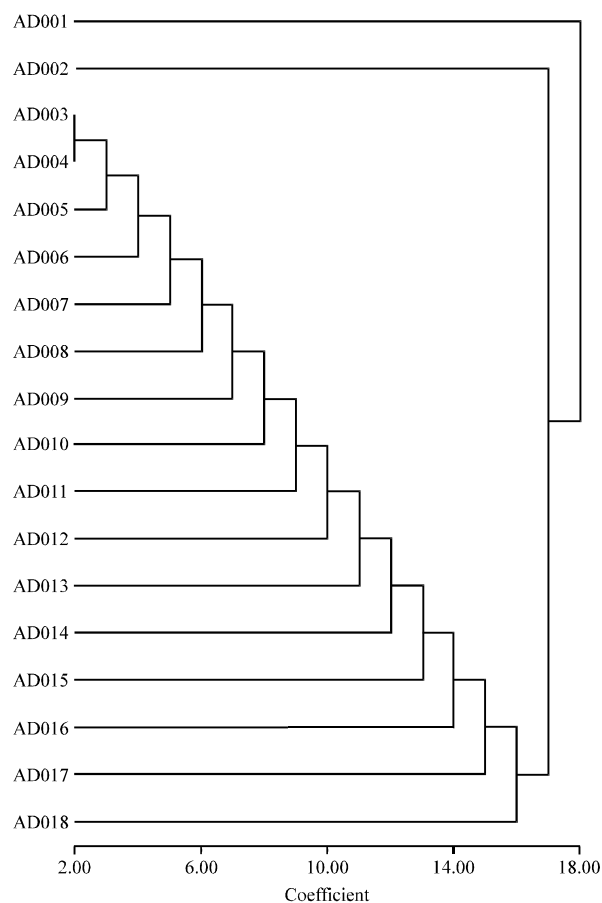


Fig. 2: A UPGMA Dendrogram of 18 melon cultivars based on RAPD analysis

showed different interrelationships based on molecular data. The result from RAPD analysis showed the samples are monomorphic and also confirms a monophyletic group (Fig. 2). Samples AD001 and AD002 showed some distinct feature at 18.00 and 16.94 similarity coefficient, also samples AD003 and AD002 appeared to have a closer relationship with each other examined with 2.06 similarity coefficient.

DISCUSSION

Range of seed sizes and colors were observed among the studied cultivars which supports Mohr (1986) that seeds of melon crops, ranged in size from very small seed size (size of tomato seeds) to large seed sizes (size of pumpkin seeds), production of white colored seeds as well as large and medium sized seeds.

DNA classification of the 18 melon cultivars showed that the genetic similarity among them was about 10.00 which suggested a wide genetic base in the local selection of melon. At 2.1 similarity coefficient samples AD015 and AD014 showed some distinct features from the morphometric evaluation in the dendrograms with 8.86

similarity coefficients. Two pairs of sample AD003 and AD017 and AD002 and AD006 showed some level of relatedness at 4.00 and 5.00, respectively, they belong to the same sub-clusters. As found in this study molecular diversity need not to be directly associated with morphological diversity as this was similarly observed with other species (Petit *et al.*, 2002). Levi *et al.* (2001), while analyzing polymorphism in five accessions of *Citrullus lanatus* var. *lanatus*, recognized a low level of polymorphism even though the cultivar studied represented a wide range of horticultural traits.

CONCLUSION

The application of RAPD seems very valuable in cultivar identification and taxonomic relationships. The extent of variation and of relatedness was revealed among the 18 melon cultivars and this can afford breeders the opportunity to make selections that can breed improved melon cultivars. However, further research needs to be done to find primers that can amplify the extracted DNA and to further characterize the melon population.

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