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Full Length Research Paper

Phylogenetic and genomic relationships in the genus *Malus* based on RAPDs

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Two separate experiments were carried out between local and exotic apple among the locally available varieties. Ten varieties of local apple (*Malus* spp.) were selected from South west Nigeria. The choice of the samples was based on observed morphological differences such as size of fruits, fruits color, flower color, leaf shape and plant height while the choice of imported exotic type was based solely on the color of the fruit. Total genomic DNA was isolated from local and exotic apples separately and assessed with RAPD markers. Nine primers generated a total of forty-six polymorphic bands, which were used to generate a UPGMA dendrogram. The dendrogram consists of a single cluster from 0 – 65% similarity coefficient. At 71%, two clusters were discerned with sample 6 and 7 having separated from the clusters. However, two samples 3 and 4 tied at 87% showing the possibility of very close relationships. Two local apples (green and red color) and one exotic apple (green color) were analyzed with 132 bands from 16 polymorphic primers. The dendrogram generated showed a closer relationships between green exotic and green local varieties of apple.

Key words: Apple, RAPD, UPGMA, polymorphic.

INTRODUCTION

Apples are members of the rose family “Rosaceae” and the genus *Malus.* They were grown and prized for their fruit by the people of ancient Rome. It is believed that the Romans took cultivated apples with them into England when they conquered the country. Petals of the flowers are pink with five petals. The ovary is epigynous or inferior, embedded in the floral cup or hypanthium. It contains 5 locules, usually 2 ovules per locule. Flowers are produced terminally from mixed buds (containing both leaves and flowers) on long shoots (Jackson, 2003). Apples have a broad spectrum uses as food; most apples are eaten raw but can be made into jellies, pies, cakes, and puddings. They can also be canned, juiced and optionally fermented to produce apple juice, cider, vinegar and pectin.

Within the last decade, technological advancement has increasingly supported the use of genetics in determining population diversity of species especially *Malus* . Molecular techniques are now available to determine the genetic architecture of wide varieties of closely related individuals. Some of these methods have been applied in revealing genetic identities and diversity in *Malus* species.

With the ever growing requirements for environmental protection and food safety in the production of high quality apples, the modern apple breeding has become more and more dependent on resistance genes (Crosby et al., 1992). The fact that apple cultivars are maintained by vegetative propagation, the monoclonal characters of apple cultivars and the large number of cultivars that have originated by mutation, indicate that intracultivar and intercultivar genetic variation is expected to be minimal. Accurate characterization of the available genetic pool is therefore important in breeding programs and essential for the protection of future property right over new cultivars.

The traditional methods for characterization and assessment of genetic variability based on morphological, physiological and agronomic traits are often not adequate, since these traits are developmentally regulated or

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influenced by the genotype x environment interaction and agronomic practices like selected rootstock or pruning. DNA markers provide opportunity for genetic characterization and allow direct comparison of different genetic material independent of environmental influences. DNA markers are also more abundant than morphological and biochemical markers and the whole genome can be assessed. Watilon et al. (1991) successfully used RFLP technique to identify cultivars and assess the inheritance of the markers obtained, while Nybon and Schaal (1990) used it to determine the paternity of seedlings. The RAPD analysis has already proved to be valuable in apple genome analysis. These markers have been used to study relationships in the genus *Malus* (Dunemann et al., 1994), for identification of apple cultivars, (Koller et al., 1993); Mulcahy et al., 1993) and apple rootstocks (Autio et al., 1998) and for paternity analysis (Harada et al., 1993; Gardiner et al., 1996). Luis et al. (2001) employed both RAPD and AFLP in discrimination and estimation of genetic similarities among apples cultivars while Zhou and Li (2000) used RAPD for the phylogenetic relationships of the closely related species of cultivated apple.

The main objective of this study was to assess the level of genetic diversity of apples that were grown in Nigeria and the similarities between this local apple from Nigeria and the exotic ones imported from abroad with a view to examining cross compatibility between them.

MATERIALS AND METHODS

Plant materials and DNA extraction

Ten samples of apple trees were selected from local species and young leaves from the apical shoot were collected from each for DNA extraction using modified protocol of Delaportal et al. (1983). At a separate experiment green and pink fruits of exotic and a green fruit of local apple were taken for total genomic DNA extraction using the same protocol.

PCR amplification

RAPD reactions are assembled and mixed to create a cocktail of 20 µl total volume per unit. 10 x Buffer (2.0 µl), $MgCl_2$, (1.6 µl), Tween 20 (2.0 µl), $DNTP_S$ (1.0 µl), Primer (4.0 µl), Tag DNA polymerase (0.2 µl), distilled water (9.2 µl) and template DNA (3.0 µl). The sample was mixed thoroughly and centrifuged briefly to bring down the contents of the tube. They were then placed in the thermocycler machine for DNA amplification. The PCR was carried out with the RAPD profile of 45 cycles preheated at 94°C for 3 min, 94°C denaturation for 30 s, 37°C for 40 s annealing and 72°C final extension for 7 min.

Gel Scoring and data analysis

Fragments that were clearly resolved on gels were scored as 1 or 0 for present or absent, respectively, while bands that could not be confidently scored were regarded as missing data. Pair wise distance (similarity) matrices were computed using sequential, hierarchical and nested (SAHN) clustering option of the NTSYS-pc version 2.02j software package (Rohlf, 1993). The program generated dendrograms, which grouped the tested lines on the basis of Nei genetic distance (Nei, 1972) using unweighted pair group method with arithmetic average (UPGMA) cluster analysis (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

Polymorphic unambiguous bands that were clearly resolved on gels were scored as present (1) or absent (0) while monomorphic, ambiguous and faint bands were ignored. A total of 16 Operon primers generated one hundred and thirty four bands for the dendrogram in Figure 1 while 9 primers generated the dendrogram in Figure 2 from a total of forty five bands. A chart in Figure 3 revealed that primer OPAC 07 was highly informative with the highest number of polymorphic bands of 13 while three primers OPAE 03, OPAC 13 and OPAF 13 showed 2 polymorphic bands each. The UPGMA dengrograms generated in Figures 1 and 2 classified the tested samples into various clusters and sub clusters at different levels of similarity coefficient.

The molecular classification of the ten apple cultivars showed that the genetic similarity among them was about 0.65 which suggests a narrow genetic base in the local selection of apple. At 0.87 similarity coefficient, two samples 3 and 4 appeared to have a closer relationship with each other than any other samples examined. This was due to the fact that although they were from different locations yet they were the only samples with small sized tree of about (5-10 m height), narrow canopy of elliptic leaves with entire margin and light green leaves while others have medium sized tree (5-12 m tall) with spreading canopy, elliptic dark green leaves with serrated margins (Manhart, 1995).

Samples 6 and 7 showed some distinct features from the morphometric evaluation and this was evident from the molecular evaluation in the dendrogram. The two samples were from Oshogbo in Osun state Nigeria but sample 6 was copiously branched from about 3 m above the soil level to the shoot top and has the smallest fruit size while sample 7 was not as branched as the former but possessed lighter flower colour different from all other samples. Two pairs of samples (1 and 8) and (5 and 10) showed different levels of relatedness at 0.72 and 0.80 similarity coefficients respectively and they belong to different subcluster.

Information on the origin of cultivated and its phylogenetic relationship with the closely related species (Dunemann et al., 1994) is now becoming more and more important for future apple breeding. Perhaps this may solve the problem of cross incompatibility experienced by some breeders in Africa especially in Nigeria where breeding of cultivated apple did not produce as big as apple imported from abroad. Generally, apple fruit quality is best in temperate climates with high intensity, warm (not hot) days and cool nights, hence the success of apple culture are self-incompatible and must be cross-pollinated with the closest neighbor to develop viable

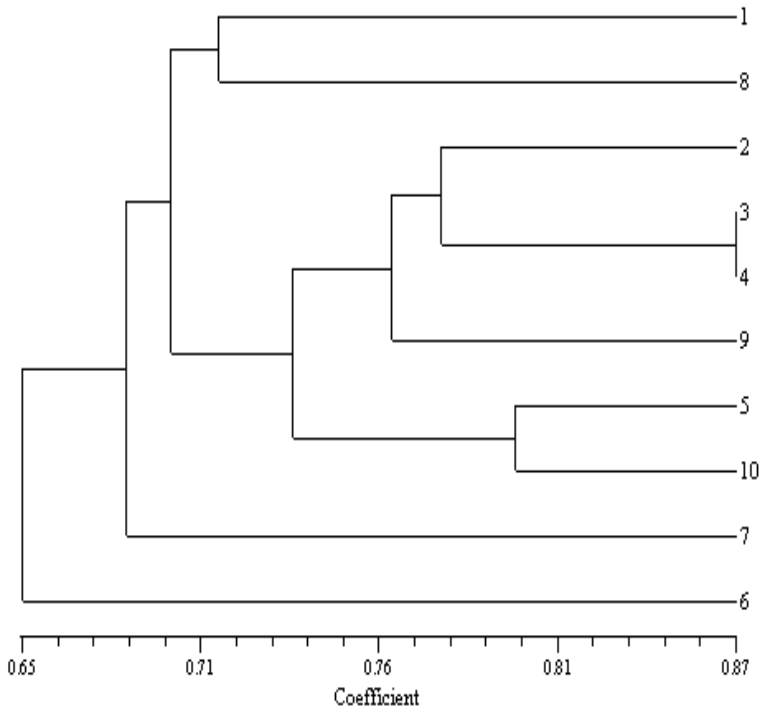

Figure 1. A UPGMA Dendrogram of 10 *Malus* samples selected from South West Nigeria based on RAPD analysis.

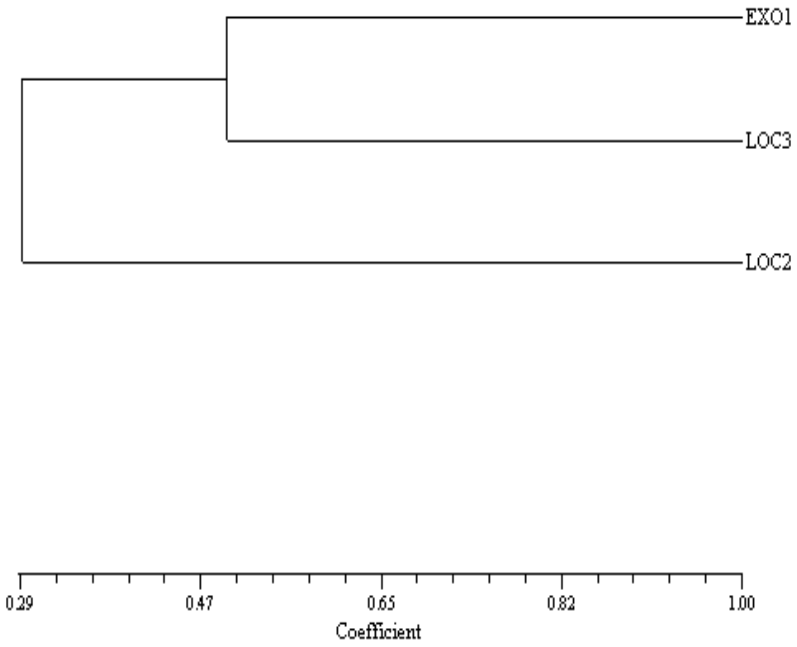

Figure 2. A UPGMA dendrogram generated from sixteen primers with one hundred and thirty four bands of RAPD marker when analyzed with two local (LOC2-red, LOC3 - green)and one exotic EXO1-green.

fruit. However, in Figure 2, the dendrogram revealed clustering of green local and green exotic apples in the same cluster. Meanwhile, clustering analysis with the samples on a primer bases revealed twelve out of sixteen primers showing the same pattern as in Figure 2. Thus, revealing possible genetic relatedness between the local

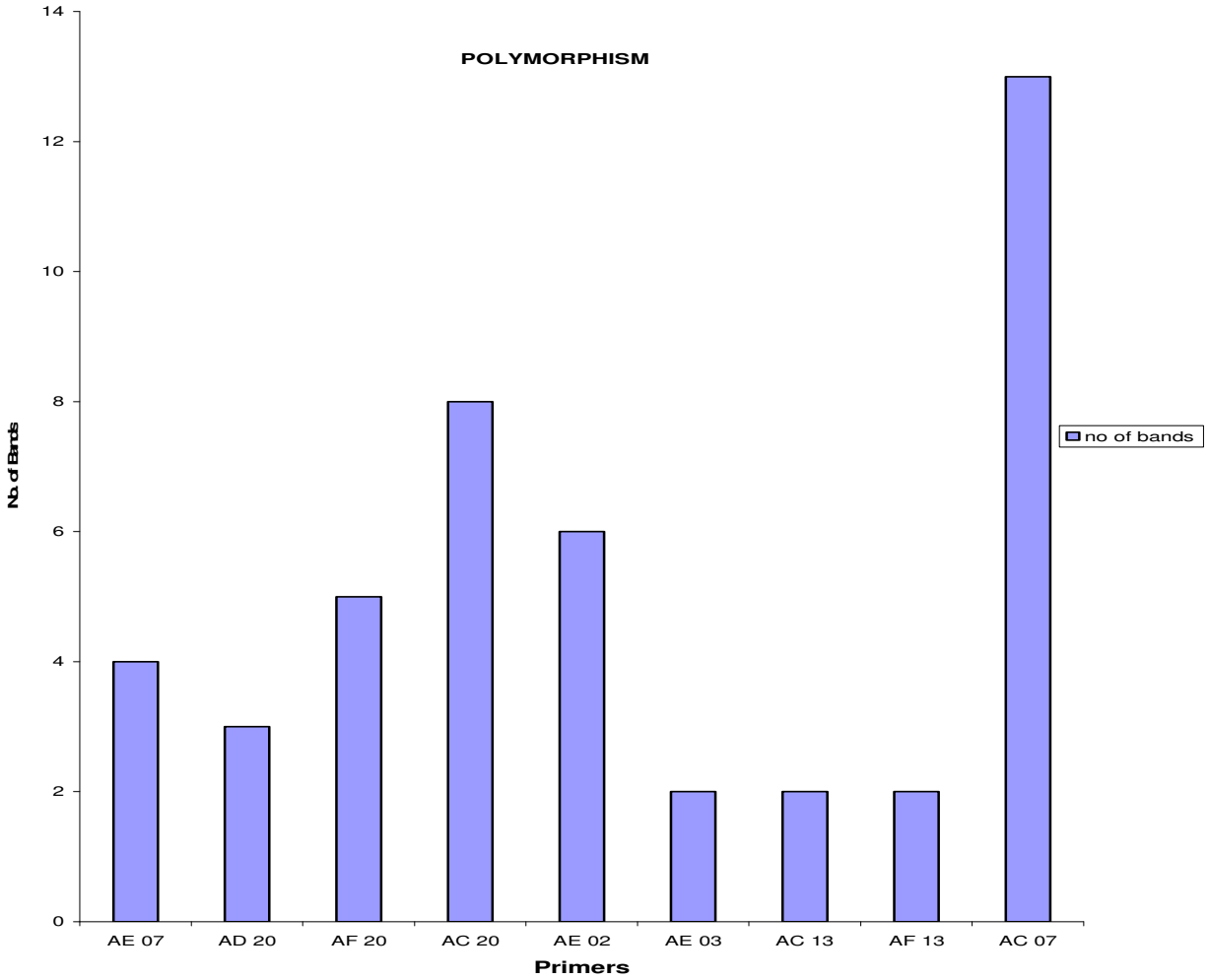

Figure 3. Number of polymorphic bands generated from each primer.

green and the exotic green apples, therefore cross compatibility may be possible between the two (Zhou and Li, 2000). In general, it has been demonstrated that RAPD analysis may be a useful tool for estimation of genetic relationship of closely related apple genotypes as reported by Dunemann et al. (1994), Koller et al. (1993) and Danladi et al. (2005)

Future breeding could be tried between green exotic and green local apples for possible cross compatibility and viable hybrids

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