FINGERPRINTING OF SWEETPOTATO GERMPLASM USING AFLP, RAPD, AND SAMPL ANALYSIS

AMINA A. MOHAMED, MERVAT M. M. EL FAR AND M. E. SAAD

Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), 9 Gamaa St. 12619, Giza, Egypt

Sweetpotato *(Ipomoea batatas*) a storage tubers root belongs to the morning-glory family is the sixth important food crop. World-wide, sweetpotato is cultivated over 9 million hectares with a total yield of 105 million tons annually (FAO, 2014). In Egypt, sweetpotato is one of the most widely grown root crops. It is grown in upper-Egypt, Nubaria, Kafr El-Sheikh and Monofia, with a total production of 320,000 tons (FAO, 2014). The distribution of sweetpotato over a wide-range of environmental conditions ranging from tropical, subtropical and moderate climate, altitudes from sea level to 2.500 meters reflect its wide range of biodiversity (The World Bank, 2011). Improving quality and yield is an important target for increasing the economic value of sweetpotato. The ability to improve productivity and agronomic characters through breeding programs depend on assessing the genetic variation of sweetpotato cultivars and their genetic relationship to other genotypes. In addition, studying of genetic diversity supports the conservation of genetic resources. On the other hand, DNA molecular markers techniques detect specific locations at the DNA level that differ among cultivars or improved species. Therefore, they play a crucial role in all aspects of plant breeding, and widely used to estimate genetic diversity. Compared to conventional phenotyping methods, molecular markers are stable in plant tissues regardless of environmental influences (Luo *et al*., 2016). DNA fingerprinting has become an important tool for assessment of biodiversity, germplasm management, identifying markers for useful traits, studying gene diversity and variation within breeding populations and differencing between plant species and cultivars.

Three different DNA-based markers including random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and selective amplification of microsatellite polymorphic loci (SAMPL) were used in the assessment of the diversity and generation of DNA fingerprints for cultivars. They are used to detect polymorphism occurring on the genome sequence level. All of these methods rely on the use of oligonuceleotide primers to produce PCR fragments using genomic DNA as a template, resulting in multi-locus banding patterns that are recorded following electrophoretic separation and visualization. A number of different molecular assays have

Egypt. J. Genet. Cytol., 45:383-401, July, 2016
Web Site (*www.esg.net.eg*)

been applied in sweetpotato including RAPD (Connolly *et al*., 1994; Zhang *et al*., 1998; He *et al*., 2006; Moulin *et al*., 2012; Lee *et al*., 2015), AFLPs (Zhang *et al*., 2000; Elameen *et al*., 2008; Liu *et al*., 2012), inter simple sequence repeats, ISSRs (Hu *et al*., 2003; He *et al*., 2007), SAMPL (Tseng *et al*., 2002) and simple sequence repeat markers, SSRs (Gichuru *et al*., 2006; Ngailo *et al*., 2016).

In this study, RAPD, AFLP and SAMPL were used to study the polymorphism among ten sweetpotato germplasm to generate fingerprint profile to each cultivar and studying the genetic relationships among these accessions. The ability of these different types of molecular marker techniques to detect and measure the genetic diversity of sweetpotato germplasm under investigation was compared.

MATERIALS AND METHODS

Plant materials

In this study two Egyptian local varieties (Abees and Mabrouka) and eight germplasm of sweetpotato *Ipomoea batatas* (L.) Lam. obtained from the International Potato Centre (CIP), Lima, Peru and selected according to their productivity, chemical characteristics, and organoleptic properties (EL-Bastawesy *et al*., 2008) were used (Table 1).

DNA extraction

Genomic DNA was extracted from young leaf materials using modified CTAB method (Doyle and Doyle, 1990). Purity and concentration of DNA was assessed by the NanoDrop ND-1000 Spectrophotometer (Thermo-Fisher, Waltham, Ma, USA).

RAPD analysis

The DNA amplification protocol was performed as described by Williams *et al*. (1990). A total of 40 arbitrary 10-mer primers (Operon Technology, Inc., Alameda, CA, USA), were used in the detection of polymorphism among sweetpotato cultivars and accessions (Table 2). PCR reactions were carried out in a total volume of 25 µl containing 20 ng genomic DNA, 200 µM dNTPs, 1 µM of primer, 1X PCR buffer, 2.5 mM $MgCl_2$, and 2 units of Taq DNA polymerase (MBI Fermentas Inc., Wisconsin, USA). Reaction was performed on a GeneAmp PCR System 9700 (PE Applied Biosystems, USA).

The PCR amplification conditions included an initial denaturation step at 94°C for 5 minutes followed by 40 cycles of denaturation for 1 minute at 94°C and one minute of annealing at 36°C and 2 minutes of extension at 72°C and succeeded by 7 minutes of incubation at 72°C. Samples were stored at 4°C till analysis

PCR products were separated on 1.4% agarose gels in TAE buffer stained by 0.5 g/ml ethidium bromide and photographed under UV light. The 1 kb DNA

ladder (Fermentas, Gene Ruler) was used as a molecular size standard.

AFLP analysis

The AFLP protocol was performed as described by Vos *et al*. (1995) using the AFLP kit system I (Invetrogen, Carlsbad, California, USA) according to the manufacturer's procedure with minor modification. The DNA (500 ng) was digested with *Eco*RI and *Mse*I perior to ligation with *Eco*RI and *Mse*I adapters to generate template for amplification. Preamplification was carried out with +1-primers each carrying one selective nucleotide (*Eco*RI + A, *Mse*I + C) using polymerase chain reaction (PCR). Selective AFLP amplification was carried out with *Eco*RI + 3 primers and *Mse*I +3 primers and the diluted PCR products from the preamplification. Primers combinations that were used are presented in Table (2). PCR products were separated by electrophoresis on a denaturing 6% polyacrylmide gel in 1 x TBE buffer (Tris-Boric acid-EDTA) at 50 W for 1.5 hr. The SILVER SEQUENCE® DNA Staining Reagents Cat # Q4132 (Promega Corporation, Wisconsin, USA) was used to detect bands in a polyacrylamide gel according to the manufacturer's protocol.

SAMPL analysis

SAMPL analysis is a modification of AFLP methodology to amplifying microsatellite loci using generic PCR primers (Witsenboer *et al*., 1997). The selective amplification uses one AFLP primer in combination with a primer complementary to microsatellite sequences primer (Table 3). The thermo cycle conditions were used as described by Vos *et al*. (1995) with minor modifications (Karimi and Kafkas, 2011) using GeneAmp PCR System 9700 (PE Applied Biosystems, USA). PCR products were separated and stained like AFLP analysis.

Data analysis

Amplified products for RAPD, AFLP and SAMPL markers were scored visually on the basis of the presence or absence of bands as '1' or '0', respectively. To compare the discriminatory power of these techniques for diversity assessment in sweetpotatoes, genetic parameters such as number of observed alleles (Na), number of effective alleles (Ne) (Hartl and Clark, 1997), Shannon index (I) as a measure of gene diversity (Shannon, 1949) were calculated by the POPGENE software version (1.32) (Yeh *et al*., 1997) and the polymorphic information content (PIC), as a value of a marker for detecting polymorphism within a population, was estimated by the Power Marker software version (3.25) (Liu and Muse, 2005) according to Botstein *et al*. (1980). Average number of alleles per locus, allele frequency (p_i), expected heterozygosity (H), effective number of alleles per locus (ne) were calculated as described by Morgante *et al*. (1994). Also, the multiplex ratio (MR), that represent the total number of loci detected per assay and the effective multiplex ratio (EMR) was defined as the average number of

polymorphic loci detected per assay were calculated, according to Powell *et al.* (1996). The marker index (MI) was used to calculate the overall utility of a marker system depending on the formula; MI = EMR x PIC (Powell *et al*., 1996; Arizio *et al*., 2009; Tonk *et al*., 2011). The relationships between the distance matrixes were analyzed by the Power Marker program according to Mantel (1967). Pairwise comparison of genotypes, based on the presence (1) or absence (0) of unique and shared polymorphic products was used to generate similarity coefficients using statistical software package STATISTICA-SPSS (Stat Soft Inc.). The similarity coefficient was used to construct a dendrogram by the un-weighted pair group method with arithmetic averages (UPGMA according to Nei and Li (1979).

RESULTS AND DISCUSSION

The PCR-based marker technologies (RAPD, AFLP and SAMPL) were used to estimate the level of polymorphisms among ten sweetpotato germplasm used in this study, to generate fingerprints to each cultivar, to estimate the genetic relationships among these genotypes and compare the ability of these different technologies to estimate genetic similarity among sweetpotato germplasm.

Polymorphism revealed by RAPD analysis

Sweetpotato germplasm were analyzed using 40 RAPD primers, out of which 18 produced high number of polymorphic and reproducible fragments. These primers generated a total of 213 discrete amplified DNA fragments in all samples, with an average of 21.3 amplified DNA fragment per accession (Table 2). The number of fragments ranged from 6 to 21 with an average of 11.8 fragments per primer. A 68.1 % of the total fragments were polymorphic with an average of 8.1% markers per primer, 14.5% per genotype which indicated a high level of polymorphism among the genotypes studied (Moulin *et al*., 2012). A maximum number of polymorphic fragments (17) were amplified with primer OPM-18 while primer OPA-10 revealed the lowest polymorphism (2) (Table 2). The size of the amplified fragments also varied with the different primers and ranged from 150 to 2300 bp. These primers clearly distinguished all of the 10 sweetpotato germplasm (Fig. 1). This was in agreement with da Silva *et al.* (2014) who analyzed 52 sweetpotato genotypes from the North east of Brazil using nine RAPD primers. These primers generated a total of 50 fragments (100% polymorphic). The highest numbers of fragments were 10. Moulin *et al.* (2012) studied fifty-nine sweetpotato samples from rural properties from Brazil and 19 from local markets using 18 primers. Out of 150 amplified bands, 145 were polymorphic with an average of 8.1 polymorphic fragments per primer. He *et al.* (2006) tested 30 RAPD primers on 108 sweetpotato accessions and obtained 218 polymorphic markers, with an average of 7.3 polymorphic bands per primer. They suggested that spontaneous mutations might be the cause for this high level of genetic variations detected in

the studied sweetpotato accessions. Gichuki *et al.* (2003) utilized 11 primers on 74 sweetpotato accessions from different regions of the world. They found 71 polymorphic markers, with a mean of six polymorphic bands per primer. Sagredo *et. al.* (1998) found that analysis of 28 cultivars from all over the world revealed polymorphic bands with all 18 RAPD primers tested. Averaging 6.9 polymorphic bands per primer, total of 124 bands were scored. These results confirm that sweetpotato exhibits high genetic variation. Based on these results, the RAPD technique is considered efficient for determining the genetic variability of sweetpotato germplasm.

Polymorphism as revealed by AFLP analysis

AFLP analysis of the sweetpotato germplasm, using the five primer combinations, gave a total of 344 selectively amplified bands, ranging in size from 50 bp to 650 bp (Fig. 2). Table (3) summarizes the number of bands amplified for different primer combinations and percentage polymorphism detected in the 10 sweetpotato germplasm. The number of amplified bands per primer combination ranged from 48 to 83 with an average of 68.8 bands per primer and 34.4 per genotype. A 71.8% of the total fragments were polymorphic with an average of 49.4 per primer. The highest percentage of polymorphism (86%) was obtained using primer combination (E-ACC/M-CTA), whereas, the lowest percentage of polymorphism (63%) was detected by primer combinations (E-ACG/M-CAG), (Table 3), confirming the high multiplex ratio produced by AFLP marker. Figure (2) illustrated AFLP profile generated by primer combination E-ACT + M-CAG. These estimates agreed with those of Zhang *et al*. (2000); He *et al.* (2006); Elameen *et al*. (2008); Wang *et al*. (2011) and Liu *et al*. (2012) who concluded that the multiplex ratio obtained from AFLP was higher than that for other techniques. Cao *et al*. (2014) analyzed 98 sweetpotato varieties from China using 17 AFLP primer combinations which revealed a total of 410 polymorphic bands with an average of 24.12 polymorphic bands per primer combination.

Polymorphism as revealed by SAMPL analysis

Two ISSR primers were used in combination with one *Mse1* primer to genotype the sweetpotato germplasm (Table 3). The method combined the high multiplex ratio of AFLP with the high level of variability of SSRs. These SAMPL primer pairs gave 85 polymorphic bands out of a total 132 bands, with an average of 65 bands per primer pair. When considering the SAMPL primer combinations in which the same *MseI* primers were used in the combination with primer number 6, based on AC repeats, it generated fewer (62 bands) but more polymorphic (70.9%) than the other one, which is based on G repeats as shown in Table (3). The size of amplification products ranged from 50 to 650 bp. Figure (2b) shows a SAMPL fingerprint obtained with

primer combination $(AC)_8$ AYG/M-CTA). Tseng *et al*. (2002) obtained greater polymorphism in sweetpotato using 12 SAMPL primer pairs to analyze the genetic relationships between 22 elite cultivars of sweetpotato [*Ipomoea batatas* (L.) Lam.] used in polycross breeding in Taiwan. They also found that SAMPL markers was efficient compared to other molecular marker methods, such as RAPD and SSR markers, in assessing the genetic relationships of sweetpotato cultivars. Tosti and Negri (2002) reported that the AFLP and SAMPL techniques appear to be more efficient than the RAPD technique in the analysis of limited genetic diversity among the cowpea landraces tested.

Genetic similarity

To estimate the genetic diversity in the evaluated germplasm, amplified data from each marker system was used for calculation of genetic distance matrices. The UPGMA algorithm was used for grouping all germplasms based on their genetic distances. Dendrograms representing most probable genetic relationships between cultivars are presented in Fig. (3A, B, C and D). The genetic similarity estimates (GSEs) obtained varied depending on the DNA marker technique used: with RAPDs, GSEs ranged from 0.655 (Abees and acc #199035.7) to 0.939 (acc #199015.14 versus acc #199026.1) (average 0.797). As for AFLPs, GSEs ranged from 0.749 (Tainung versus Japones Tresmecino) to 0.936 (acc #199015.14 versus acc #199026.1) (average 0.843). SAMPL markers revealed GSEs which ranged from 0.742 (Abees versus acc #199062.1) to 0.928 (acc #199015.14 versus acc #199026.1) (average 0.835). All analysis confirmed the appreciable genetic similarity present in sweetpotato germplasm. However some germplasm evaluated in this study were shown to be very closely related and the highest degree of genetic similarity was observed between (acc #199015.14 and acc #199026.1) within all the three markers used. The GSEs from combined data for three markers presented in Table (4) summarizes all information obtained.

The mean genetic similarity of 0.797 obtained in our study is not very different from the value of 0.691 found among sweetpotato cultivars in Taiwan (Tseng *et al.,* 2002), and much closer to the value of 0.709 among Tanzanian sweetpotato accessions (Elameen *et al.,* 2008) and significantly higher than the value found when examining accessions from South America (0.588) (Zhang *et al.*, 1999). The dendrograms obtained using the data from each marker system and the data combined displayed similar, but not identical, germplasm distribution. The dendrogram for RAPD (Fig. 3-A) was similar to those of AFLP and SAMPL dendrograms with some differences in the positioning of some accessions. The dendrograms classified the ten sweetpotato genotypes into two main clusters. Cultivars Abees and Mabruka were grouped separately in the first cluster based on AFLP and SAMPL markers, while Abees was allocated to a specific

group based on RAPD. Four major clades were formed in the second main cluster. The first clade shared acc #199015.14, acc #199026.1 and Beuregard. Tainung and acc #199035.7 assembled together in the second clade. The Fourth clade shared acc #199062.1 and Japones Tresmecino, demonstrating that there was good correspondence between the results generated with these three types of markers. Construction of the general dendrogram using the combined data (Fig. 3-D) using the three sets of molecular markers was very similar to those obtained separately with each marker and the dendrograms from AFLP and SAMPL data were the most similar to the general dendrogram. The phylogenetic tree dendrogram showed that the 10 germplasm could be classified into two major groups. The first group consisted of two accessions. The second group consisted of 3 subgroups acc #199015.14; acc #199026.1 and Beuregard were congregated together while acc #199015.14 and acc #199026.1 were closer. Second subgroup had three genotypes (Santo Amaro, Tainung and acc # 199035.7), while the third subgroup consisted of #199062.1 and Japones Tresmecino.

In general, observed groups were expected, based on previous knowledge of the geographical origin of studied cultivars. Liu *et al.* (2012) found that the AFLP-based genetic distance ranged from 0.0546 to 0.5709 with an average of 0.3799. The dendrogram based on AFLP markers indicated that sweetpotato germplasm coming from the same regions or sharing the same parents were clustered in the same groups. A moderate mean genetic distance of 0.58 was also found among sweetpotato genotypes from China, the world's leading producer (He *et al.*, 2006). In contrast, Gichuru *et al.* (2006) observed low diversity among some East African sweetpotato genotypes probably because of the small number of accessions collected from few districts and also fewer number of SSR primers (four primer pairs) used in the study. Similar low diversities were observed among the sweetpotato genotypes from Tanzania (Elameen *et al*., 2008), Papau New Guinea (Zhang *et al*., 1998), and the United States (He *et al*., 1995). The relatively high genetic diversity of sweetpotato in Uganda can be attributed to their self-incompatibility leading to chance seedlings in farmers' fields and vegetative propagation of the crop and directed selection in the crop for various uses such as human food, livestock feed, and poultry feed coupled with new introductions and mutations. In cluster analysis of Tanzanian elite sweetpotato genotypes for resistance to sweetpotato virus disease and high dry matter content, Tairo *et al.* (2008) observed two major groups with a low genetic similarity of 0.52. In addition, significant differences between genotypes and genetic distance ranging from 0.26 to 0.80 were reported during morphological characterization of eight genotypes of *Solanum retroflexum* (Jacoby *et al*., 2003). A cluster analysis using morphological and simple sequence repeat markers separated some Kenyan sweetpotato genotypes into two major groups (Karuri *et al*., 2010).

Comparisons between marker systems

RAPD, AFLP, and SAMPL techniques are useful tools for assessing genetic similarity in sweetpotato, but the degree of resolution depends on the technique. Each marker system was able to discriminate among the materials analyzed. Comparative analysis presented in Table (5) summarizes all information obtained. The total number of assays was 18, 5 and 2 primer combinations for RADP, AFLP and SAMPL, respectively. The total number of bands ranged from 132 for SAMPL to 344 for AFLP. An average number of 8.1 polymorphic bands per assay unit were identified for RAPD, while for AFLP, this number increased to 49.4. The higher marker index value observed for AFLP in comparison to RAPD and SAMPL is the result of a relatively high multiplex ratio. The effective number of alleles per locus (Ne) was quite similar for each marker system, but owing to the differences in the effective multiplex ratio values the marker index was higher for the AFLP and SAMPL systems than that for the RAPD system.

All markers showed a high degree of similarity in dendrogram topology, though with some difference in the positioning of some genotypes of the subcluster. The dendrograms constructed using RAPD, AFLP and SAMPL were correlated using the Mantel test (Mantel, 1967), giving a correlation coefficient of 0.46 (RAPD and AFLP), 0.56 (SAMPL and AFLP) and 0.57 (SAMPL and RAPD) (Table 4), thus showing a moderate degree of correlation between the results obtained with the three types of markers. Teulat *et al*. (2000) reported the comparison of the four molecular marker systems (AFLP, SAMPL, ISSR and RAPD) revealed SAMPL to be the best as it generated the highest percentage of polymorphism and high MI. The capability of SAMPL markers lies in the detection of hyper variability in the microsatellite region. The measure of overall efficiency of a marker system remains the MI which is highest for SAMPL and the AFLP and SAMPL techniques appear to be more useful than the RAPD technique in the analysis of limited genetic diversity among the cowpea landraces tested reported by Tosti and Negri (2002). However Costa *et al*. (2016) found that AFLP seemed to be the best suited molecular assay for fingerprinting and assessing genetic relationship among genotypes of *Dactylis glomerata* when compared with RAPD and ISSR. The results demonstrated that RAPD, AFLP and SAMPL markers could be successfully utilized for genetic classification, DNA fingerprinting and the elimination of duplicates in sweetpotato germplasm collections preserved in gene banks.

SUMMARY

The ability to improve productivity and agronomic traits of sweetpotato through breeding programs depends on assessing the genetic variation of their germplasm and genetic relationship to other genotypes. In addition, studying genetic diversity supports the conservation of genetic resources. In this study, three

different DNA-based markers, random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and selective amplification of microsatellite polymorphic loci (SAMPL) were used for fingerprinting and detecting genetic variation for ten germplasm of sweetpotato. Results indicated that RAPD assays using 18 primers produced 213 bands, 145 of which were polymorphic with a percentage of 68.1%. AFLP using five primers yielded 344 amplified products with a percentage of 71.8% polymorphism. SAMPL using two primers combinations amplified 132 bands in which 85 being polymorphic representing 64.4%. Genetic relationship was estimated using Dice's coefficient values between different accessions, ranging from 0.655 to 0.939 in RAPD, 0.749 to 0.936 in AFLP, and 0.742 to 0.928 for SAMPL. The UPGMA algorithm was used for grouping all germplasm based on their genetic distances. In total, the three molecular marker systems were compared on the basis of multiplex ratio, marker index and average heterozygosity and revealed that AFLP was the best-suited molecular assay for fingerprinting and assessing genetic relationships. All analysis confirmed the relatively high genetic diversity present in sweetpotato germplasm used. Also, distinct DNA fingerprinting profile could be obtained with all the three molecular marker systems. These results clearly indicate the usefulness of DNA fingerprinting for the identification of sweetpotato germplasm, and their potentiality to eliminate accessions duplicates from gene banks around the world.

ACKNOWLEDGMENTS

The authors thank the International Potato Centre (CIP), Lima, Peru, for providing the sweetpotato genotypes.

RFERENCESE

Arizio, C. M., N. Hompanera, E. Y. Suarez and M. M. Manifesto (2009). Genotypic identification and diversity evaluation of a sweet potato (*Ipomoea batatas* (L). Lam) collection using microsatellites. Plant Genetic Resources, 7: 135-138.

Botstein, D., R. L. White, M. Skolnick and R. W. Davis (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics, 32: 314-331.

Cao, Q. H., J. Li. A. Tang, W. Gruneberg, K. Huamani and D. Ma (2014). Ploidy level and molecular phylogenic relationship among novel *Ipomoea* interspecific hybrids. Czech J. Genet. Plant Breed., 50: 32-38.

Connolly, A. G., I. D. Godwin, M. Cooper and I. H. DeLacy (1994). Interpretation of randomly amplified polymorphic DNA marker data for fingerprinting sweet potato (*Ipomoea batatas* L.)

genotypes. Theor. Appl. Genet., 88: 332-336.

Costa, R., G. Pereira., I. Garrido, M. M. Tavares-de-Sousa and F. Espinosa (2016). Comparison of RAPD, ISSR and AFLP molecular markers to reveal and classify orchardgrass (*Dactylis glomerata* L.) germplasm variations. PloS one, 11: 1-15.

da Silva, A. V. C., L. N. T. Andrade, A. R. C. Rabbani, M. U. C. Nunes and L. R. Pinheiro (2014). Genetic diversity of sweet potatoes collection from North-eastern Brazil. African Journal of Biotechnology, 13: 1109-1116.

Doyle, J. J. and J. L. Doyle. (1990) Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.

Elameen, A., S. Fjellheim, A. Larsen, O. A. Rognli, L. Sundheim, S. Msollaand and S. S. Klemsdal (2008). Analysis of genetic diversity in a sweet potato (*Ipomoea batatas* L.) germplasm collection from Tanzania as revealed by AFLP. Genetic Resources and Crop Evolution, 55: 397-408.

EL-Bastawesy, A., A. L. Hareedy and M. M. M. El-Far. (2008). Chemical and technological evaluation of some sweetpotato varities. Arab Univ. J. Agric. Sci., 16: 87-96.

FAO, http://faostat.fao.org. (2014).

Gichuki, S. T., M. Berenyi, D. Zhang, M. Hermann, J. Schmidt, J. Glössl and K. Burg (2003). Genetic diversity in sweetpotato [*Ipomoea batatas* (L.) Lam.] in relationship to geographic sources as assessed with RAPD markers. Genetic Resources and Crop Evolution, 50: 429-437.

Gichuru, V., V. Aritua, G. W. Lubega, R. Edema, E. Adipala and P. R. Rubaihayo (2006). A preliminary analysis of diversity among East African sweetpotato landraces using morphological and simple sequence repeats (SSR) markers. In II Inter. Symp. on Sweetpotato and Cassava: Innovative Tech. for Commercialization, 703: 159-164.

Hartl, D. L. and A. G. Clark (1997). Principles of Population Genetics (Vol. 116). Sunderland: Sinauer Associates.

He, G., C. S. Prakash and R. L. Jarret. (1995). Analysis of genetic diversity in a sweetpotato (*Ipomoea batatas*) germplasm collection using DNA amplification fingerprinting. Genome, 38: 938-945.

He, X. Q., Q. C. Liu, K. Ishiki, H. Zhai, and Y. P. Wang. (2006). Genetic diversity and genetic relationships among Chinese sweetpotato landraces revealed by RAPD and AFLP markers. Breeding Science, 56: 201-207.

He, X. Q., Q. C. Liu, K. Ishiki, H. Zhai, and Y. Wang (2007). ISSR analysis of genetic diversity and relationships among sweet potato (*Ipomoea batatas*) landraces in China. Plant Genetic Resources Newsletter (Bioversity International/FAO). 150: 35-1.

Hu, J., M. Nakatani, A. G. Lalusin, T. Kuranouchi and T. Fujimura (2003). Genetic analysis of sweetpotato and wild relatives using inter-simple sequence repeats (ISSRs). Breeding Science, 53: 297-304.

Jacoby, A., M. T. Labuschagne and C. D. Viljoen (2003). Genetic relationships between Southern African Solanum retroflexum Dun. and other related species measured by morphological and DNA markers. Euphytica, 132: 109-113.

Karimi, H. R. and S. Kafkas (2011). Genetic relationships among Pistacia species studied by SAMPL markers. Plant Systematics and Evolution, 297: 207-212.

Karuri, H. W., E. M. Ateka, R. Amata, A. B. Nyende, A. W. T. Muigai, E. Mwasame and S. T. Gichuki (2010). Evaluating diversity among Kenyan sweet potato genotypes using morphological and SSR markers. Int. J. Agric. Biol., 12: 33-38.

Lee, H. M., Y. Park, T. H. Jun, S. W. Kwon, I. S. Choi, Y. C. Kim, R. Gupta, M. N. Chung, S. H. Kim, P. Yan, Y. Wang and S. T. Kim (2015). Direct sequencing of RAPD products provides a set of SCAR markers for discrimination of sweet potato cultivars. Plant Omics, 8: 195-200.

Liu, K. and S. V. Muse (2005). Power Marker: an integrated analysis environment for genetic marker analysis. Bioinformatics, 21: 2128-2129.

Liu, D. G., Z. H. A. O. Ning, Z. H. A. I. Hong, X. X. Yu, J. I. E. Qin, L. J. Wang and Q. C. Liu (2012). AFLP fingerprinting and genetic diversity of main sweetpotato varieties in China. Journal of Integrative Agriculture, 11: 1424-1433.

Luo, K., L. Hui-xiang, W. Zheng-dan, W. Xue-li, Y. Wang, T. Dao-bin, W. Ji-chun and Z. Kai (2016). Genetic diversity and population structure analysis of main sweet potato breeding parents in Southwest China. Scientia Agricultura Sinica, 49: 593-608

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. Cancer research, 27: 209-220.

Morgante, M., A. Rafalski, P. Biddle, S. Tingey and A. M. Olivieri (1994). Genetic mapping and variability of seven soybean simple sequence repeat loci. Genome, 37: 763-769.

Moulin, M. M., R. Rodrigues, L. S. A. Gonçalves, C. P. Sudré and M. G. Pereira (2012). A comparison of RAPD and ISSR markers reveals genetic diversity among sweet potato landraces (*Ipomoea batatas* (L.) Lam.). Acta Scientiarum Agronomy, 34: 139-147.

Nei, M. and W. Li (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA, 76: 5269-5273

Ngailo, S., H. Shimelis, J. Sibiya, B. Amelework and K. Mtunda (2016). Genetic diversity assessment of Tanzanian sweetpotato genotypes using simple sequence repeat markers. South African Journal of Botany, 102: 40-45.

Powell, W., M. Morgante, C. Andre., M. Hanafey, J. Vogel, S. Tingey and A. Rafalski. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding, 2: 225-238.

Sagredo, B., P. Hinrichsen, H. López, A. Cubillos and C. Muñoz (1998). Genetic variation of sweetpotatoes (*Ipomoea batatas* L.) cultivated in Chile determined by RAPDs. Euphytica, 101: 193-198.

Shannon, C. E. (1949). Communication theory of secrecy systems. Bell System Technical Journal, 28: 656-715.

Tairo, F., E. Mneney and A. Kullaya (2008). Morphological and agronomical characterization of sweet potato [*Ipomoea batatas* (L.) Lam.] germplasm collection from Tanzania. African Journal of Plant Science, 2: 077-085.

Teulat, B., C. Aldam, R. Trehin, P. Lebrun, J. H. A. Barker, G. M. Arnold, A. Karp., L. Baudouin and F. Rognon (2000). An analysis of genetic diversity in coconut (*Cocos nucifera*) populations from across the geographic range using sequence-tagged microsatellites (SSRs) and AFLPs. Theor. Appl. Genet., 100: 764-771.

The World Bank (2011). World Development Report: Conflict, Security and Development. The International Bank for Reconstruction and Development TheWorld Bank. Washington (USA), 416.

Tonk, F. A., R. R. A. Giachino, C. Sönmez, S. Yüce, E. Bayram, I. Telci and M. A. Furan (2011). Characterization of various *Hypericum perforatum* clones by hypericin and RAPD analyses. Int. J. Agric. Biol, 13: 31-37.

Tosti, N. and V. Negri (2002). Efficiency of three PCR-based markers in assessing genetic variation among cowpea (*Vigna unguiculata* subsp.

unguiculata) landraces. Genome, 45: 268-275.

Tseng, Y. T., H. F. Lo and S. Y. Hwang (2002). Genotyping and assessment of genetic relationships in elite polycross breeding cultivars of sweet potato in Taiwan based on SAMPL polymorphisms. Botanical Bulletin of Academia Sinica, 43: 99-105.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman and M. Kuiper (1995). AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research, 23: 4407-4414.

Wang, Z., L. Jun, L. Zhongxia, H. Lifei, C. Xinliang, F. Boping, L. Yujun, C. Jingyi and Z. Xiongjian (2011). Characterization and development of EST-derived SSR markers in cultivated sweetpotato (*Ipomoea batatas*). BMC Plant Biology, 11: 139-148.

Williams, J. G., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research, 18: 6531-6535.

Witsenboer, H., R. W. Michelmore and J. Vogel (1997). Identification, genetic localization and allelic diversity of selectively amplified microsatellite polymorphic loci in lettuce and wild relatives (*Lactuca* spp). Genome, 40: 923-936.

Yeh, F. C., R. C. Yang, T. B. Boyle, Z. H. Ye and J. X. Mao (1997). POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada, 10.

Zhang, D. P., D. Carbajulca, L. Ojeda, G. Rossel, S. Milla, C. Herrera and M. Ghislain (1999). Microsatellite analysis of genetic diversity in sweetpotato varieties from Latin America. CIP Program Report, 2000: 295-301.

Zhang, D., J. Cervantes, Z. Huamán, E. Carey and M. Ghislain (2000). Assessing genetic diversity of sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars from tropical America using AFLP. Genetic Resources and Crop Evolution, 47: 659-665.

Zhang, D., M. Ghislain, Z. Huamán, A. Golmirzaie and R. Hijmans (1998). RAPD variation in sweetpotato (*Ipomoea batatas* (L.) Lam) cultivars from South America and Papua New Guinea. Genetic Resources and Crop Evolution, 45: 271-277.

Table (1): The sweetpotato genotypes were used in the study and their country of origin.

S. code	CIP accession no.	Genotypes	Country of origin
1	-	ABEES	Egypt
2	-	MABROUKA	Egypt
3	440132	BEAUREGARD	USA
4	420009	JAPONES TRESMECINO	Peru
5	400011	SANTO AMARO	Brazil
6	440189	TAINUNG 64	Taiwan
7	199015.14	-	Peru
8	199035.7	-	Peru
9	199062.1	-	Peru
10	199026.1	-	CIP

Table (2): List of RAPD primers, total number of bands, number of polymorphic bands and percentage of polymorphism.

Primer	Sequence (5´-3´)	Total no. of bands	Polymorphic bands	Percentage of polymorphism (%)
OPA-10	GTGATCGCAG	7	2	29
OPB-03	CATCCCCCTG	6	4	67
OPB-16	TTTGCCCGGA	11	8	73
OPB-17	AGGGAACGAG	13	9	69
OPB-18	CCACAGCAGT	14	9	64
OPB-19	ACCCCCGAAG	10	8	80
OPB-20	GGACCCTTAC	13	11	85
OPC-04	CCGCATCTAC	19	13	68
OPC-05	GATGACCGCC	10	5	50
OPC-07	GTCCCGACGA	16	13	81
OPC-09	GTCCCGACGA	9	6	67
OPC-16	CACACTCCAG	15	8	53
OPC-17	TTCCCCCCAG	7	3	43
OPC-20	ACTTCGCCAC	21	13	62
OPD-06	ACCTGAACGG	6	5	83
OPG-02	TCTCCCTCAG	6	5	83
OPM-01	GTTGGTGGCT	10	6	60
OPM-18	CACCATCCGT	20	17	85
Total		213	145	68

Table (3): Primer combination sequences and Levels of polymorphism for AFLP and SAMPL analysis.

Serial no.	Primer comb. Sequence	Total no. of bands	Monomorphic Bands	Polymorphic bands	% of Polymorphism
1	E-ACG/M-CAG	75	28	47	63.0%
2	E-AAG/M-CTA	60	21	39	65.0%
3	E-ACC/M-CTA	78	11	67	86.0%
4	E-AAC/M- CAG	83	25	58	70.0%
5	E-AGG/M-CTT	48	12	36	75.0%
Total	**(5 primers)**	**344**	**97**	**247**	**71.8**
6	$(AG)_8$YG/M-CTA	62	18	44	70.9 %
7	$(AC)_8$YG/M-CTA	70	29	41	58.6 %
Total	**(2 primers)**	**132**	**47**	**85**	**64.4 %**

Table (4): Genetic similarity matrix among the 10 sweetpotatoes as computed according to Nei and Li's coefficient from combined data.

Genotypes	Matrix File Input								
	Mabrouka	Abees	199015.14	199026.1	Beauregard	Santo Amaro	Tainung	199035.7	199062.1
Abees	.803								
199015.14	.791	.762							
199026.1	.784	.760	***.936***						
Beauregard	.778	.765	.798	.827					
Santo Amaro	.763	.752	.775	.786	.800				
Tainung	.784	.757	.786	.788	.815	.818			
199035.7	.775	***.739***	.792	.805	.810	.796	.817		
199062.1	.774	.761	.777	.800	.793	.782	.767	.811	
420009	.772	.764	.783	.785	.781	.774	.783	.793	.847

Table (5): Levels of polymorphism and comparative information obtained with RAPD, AFLP and SAMPL markers.

Parameter	Value		
	RAPD	AFLP	SAMPL
Number of assay units	18 primer	5 primer combinations	2 primer combinations
Total number of bands (Multiplex ratio)	213.00	344.00	132.00
Number of polymorphic bands	145.00	247.00	85.00
Polymorphism% per assay	68.10	71.80	64.40
Effective multiplex ratio (EMR)	8.00	49.40	42.50
Number of observed alleles (Na)	1.72±0.45	1.65 ± 0.48	1.65c ± 0.48
Effective number of alleles per locus (Ne)	1.47±0.38	1.42 ± 0.38	1.40 ± 0.37
Polymorphic information content (PIC)	0.22	0.19	0.19
Marker index (MI)	1.60	9.39	8.08
Shannon index (I)	0.41	0.36	0.35

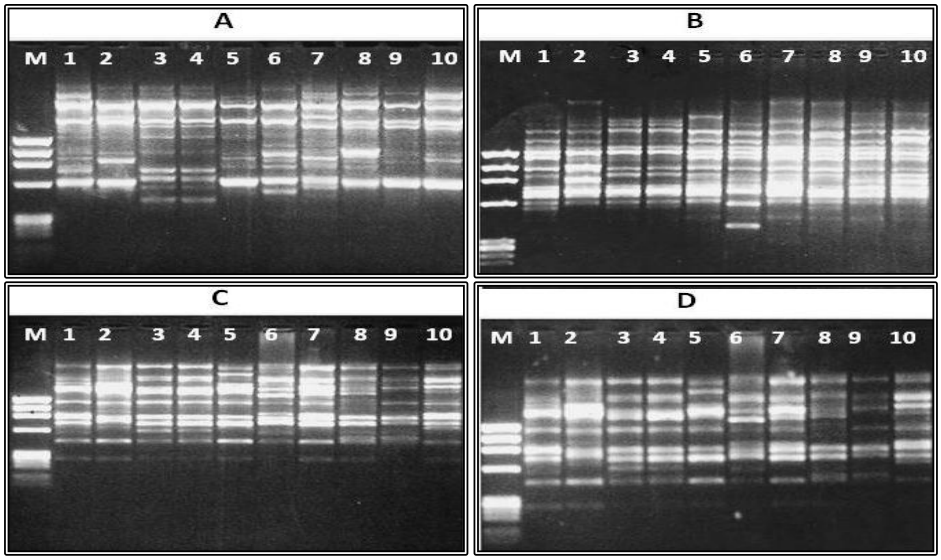

Fig. (1): RAPD patterns profile of the ten sweetpotato genotypes as descriped by different RAPD primers (M18, C4, S3 and S5, respectively). M = 1 kb DNA marker, 1-10 sweetpotato germplasm number according to the list in Table (1).

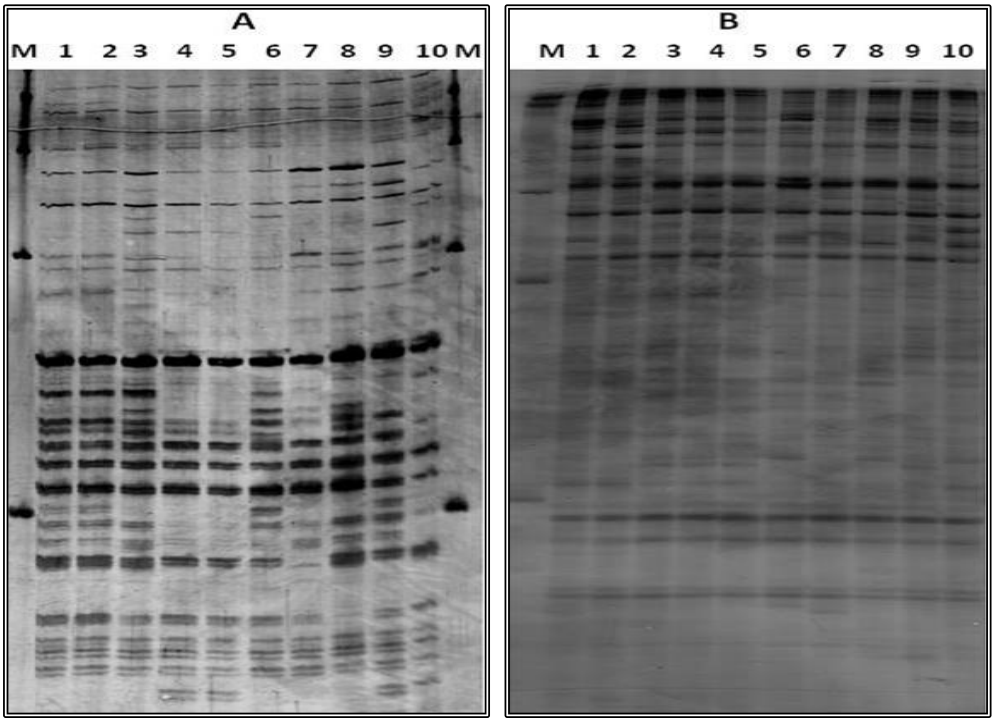

Fig. (2): PCR patterns profile of the ten sweetpotato genotypes as described by different A) AFLP combination E-ACG/M-CAG and B) SAMPL combination $(AC)_8$YG/M-CTA. M = 100 b DNA marker, 1-10 sweetpotato germplasm number according to the list in Table (1).

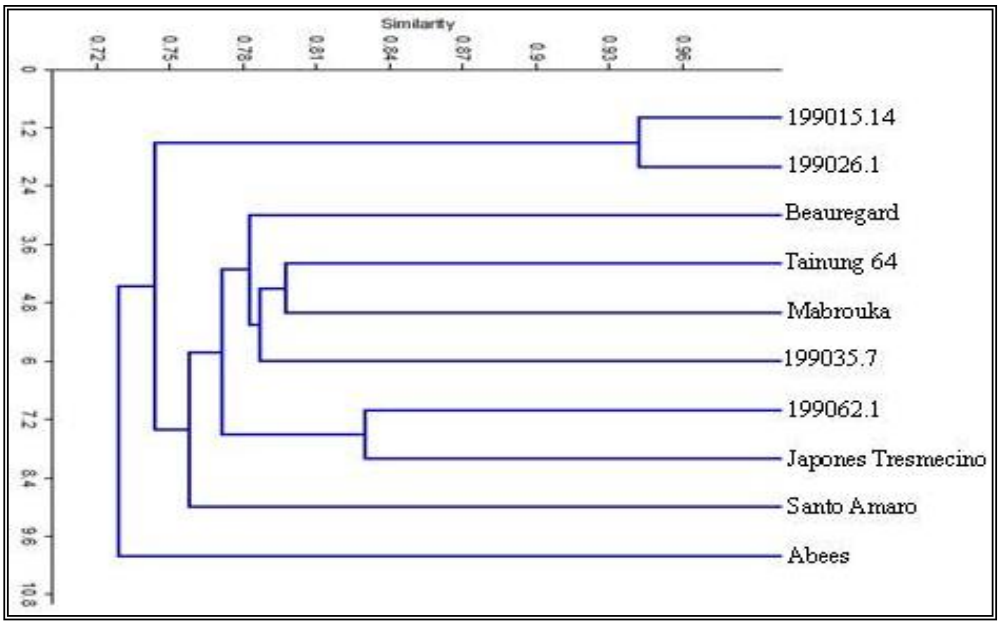

Fig. (3-A): Dendrogram for the 10 sweetpotato germplasm using UPGM according to Nei and Li's coefficient (Nei and Li, 1979). Constructed from the RAPD data.

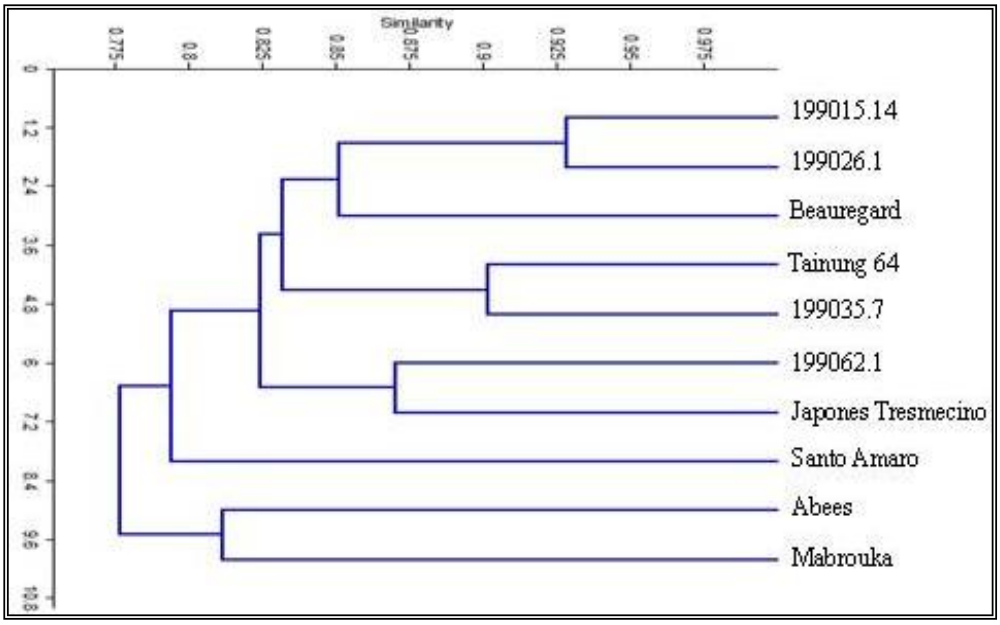

Fig. (3-B): Dendrogram for the 10 sweetpotato germplasm using UPGM according to Nei and Li's coefficient (Nei and Li, 1979). Constructed from the AFLP data.

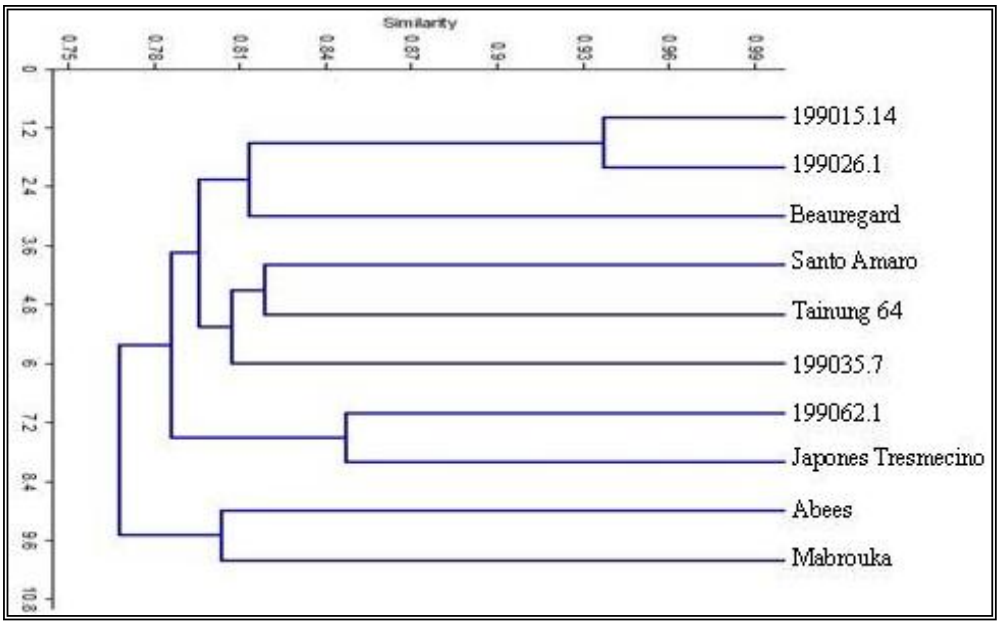

Fig. (3-C): Dendrogram for the 10 sweetpotato germplasm using UPGM according to Nei and Li's coefficient (Nei and Li, 1979). Constructed from the SAMPL data

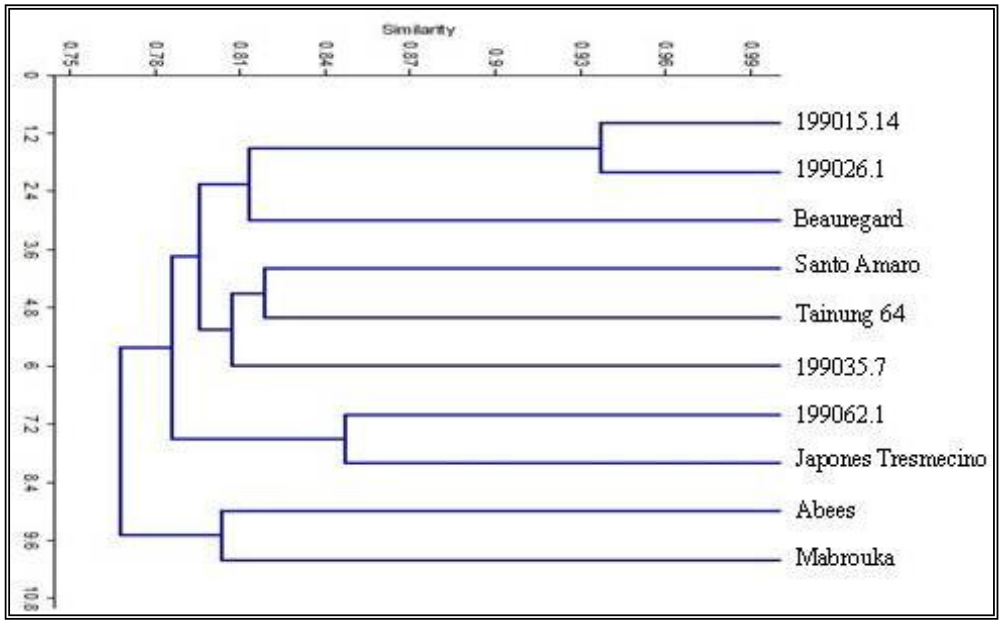

Fig. (3-D): Dendrogram for the 10 sweetpotato germplasm constructed from the combined data of AFLP, RAPD and SAMPL markers using (UPGMA) according to Nei and Li's coefficient.