# GENETIC CHARACTERIZATION OF THREE EGYPTIAN SWEET POTATO GENOTYPES BASED ON MORPHO-AGRONOMIC AND MOLECULAR MARKERS

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ret potato (*Ipomoea batatas* L. [Lam.]); one of the leading tuber crop belonging to Convulvulaceae family, is an important food crop particularly in the developing countries (Scott et al., 2000). It is grown in a wide range of agroecologies ranging from tropical, subtropical and moderate areas (Manrique and Hermann, 2000). In many African countries it forms a large part of the people's food due to its yield and nutritional value high (Bovell-Benjamin, 2007). It is a good source of energy, vitamins, riboflavin, thiamine and minerals (Woolfe, 1992). Sweet potato ranks the sixth among food crops in annual production in the world (FAO, 2014). In Egypt, it is widely grown in Nubaria. Kafr El-Sheikh. Monofia and Upper-Egypt with a total production of 320,000 tons (FAO, 2014). Only the two cultivars Abees and Mabrouka were widely grown in Egypt, thus there are not enough genetic resources causing genetic erosion. On the other hand, the genetic variations and relationships among these genotypes are poorly understood and not fully characterized, which limited their

usage in improvement of this important crop.

Success of any breeding program depends on the wide genetic variability in the available genetic resources. Thus, the first step in sweet potato improvement is to characterize and assess the genetic variation aiming to preserve of genetic materials and develop new improved varieties.

Conventionally, morphological and agronomic characters had been used for sweet potato characterization. Sweet potatoes vary in their growth habit, leaf size and shape, root flesh color, level of pigmentation and other morphological characters. Also, agronomic characters (i.e. storage root size and shape, number of root/plant, yield/plant and average root weight) vary significantly. Different morphological and biochemical markers have been used in sweet potato studies for determination of the amount and distribution of genetic variations (Das and Naskar, 2008; Yada et al., 2010; Elameen et al., 2011; Senanayake et al., 2013; Sanoussi et al., 2016). Because all of these genetic markers strongly influenced by the environment, they did not give consistent results.

On the other hand, with the advent of Polymerase Chain Reaction (PCR), DNA-based techniques such as Random Amplified Polymorphic DNA (RAPD) have been widely used in genetic studies of sweet potato for characterization, identification and determination of genetic variations at the molecular level (Zhang et al., 1998; Gichuki et al., 2003; He et al., 2006; Lin et al., 2009; Moulin et al., 2012; da Silva et al., 2014). RAPD marker detects genetic variations at the DNA level that differ between cultivars and plant species. It is widely used to estimate genetic diversity, where it is stable in plant tissues regardless of environmental effects compared to conventional markers (Luo et al., 2016).

The present study aimed to characterize three Egyptian genotypes of sweet potato; the two common cultivars (Abees and Mabrouka) as well as a new hybrid (Gendawy), using morpho- agronomic and chemical markers, in addition to assess the genetic diversity in these genotypes at molecular level using RAPD markers.

## MATERIALS AND METHODS

The present study was carried out at the Department of Vegetable Breeding Research, Horticulture Research Institute, Agriculture Research Center, Egypt and the Laboratory of Genetics Department, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh, Egypt.

## **Plant material**

Three Egyptian sweet potato genotypes; the two common cultivars (Abees and Mabrouka) as well as a new hybrid (Gendawy), were used in this study.

# Morphological and agronomic data collection

The three genotypes were grown in the field during two successive seasons (2014 and 2015) in randomized complete block design with three replications. Recommended cultural practices were followed and harvest was done at full maturing. Morphological and agronomic data were collected. Observations on morphological characters were recorded 90 days after planting. After harvest, storage root characters and yield data were recorded.

### Chemical analysis

The yielded roots were dried and grinned for chemical analysis. The percentage of nutritional characters (moisture, total carbohydrates, carotenes, Vitamin-C and protein) were measured using the standard methods of A.O.A.C. (2005).

#### **RAPD** analysis

Genomic DNA was extracted from fresh young leaves of each sweet potato genotype using Cetyl Trimethyl Ammonium Bromide (CTAB)-based method according to Saghai-Maroof *et al.* (1984).

Nine 10-mer primers (Bio Basic Inc, Canada) were screened for studying genetic diversity among the three genotypes. The sequences of these primers are given in Table (3). The PCR amplification was performed in a 25 µl reaction volume including 2 µL of 40 ng of template DNA. Final concentrations were 1 x buffer ( $Mg^{2+}$ free), 1.5 mM MgCl<sub>2</sub>, 200 µM dNTPs mix, 0.8 µM primer, 1 U Taq DNA Polymerase (ROVALAB, Germany). Amplifications were carried out in a thermal cycler programmed for the initial denaturation at 94°C for 5 min, followed by 35 cycles consisting of 30 sec of denaturation at 94°C, 45 sec of annealing at 30°C and 1.5 min of extension at 72°C. The program was ended with a final extension step at 72°C for 2 min. Amplification products were separated on 1.5% agarose gel, stained with ethidium bromide and visualized under ultraviolet light. The molecular size of the amplified products was determined against 50 bp DNA Ladder (Cat-no: 300003, GeneON).

# Data analysis

The quantitative data of the morpho-agronomic and chemical characters were subjected to analysis of variance (ANOVA) with randomized complete block design. Means were compared by least significant difference (LSD) at a significance level of 0.05.

Data generated from RAPD banding patterns of the nine primers were introduced to SPSS package program according to binary values of (1) for present and (0) for absent band. The genetic distances among the genotypes were assessed based on Jaccard's similarity coefficient (Jaccard, 1901). The phylogenetic relationship among genotypes was conducted based on the similarity coefficients of RAPD primers data using the UPGMA (Unweighted pair group mean average) method (Nei, 1973).

# **RESULTS AND DISCUSSION**

# Morphological characterization and agronomic evaluation

Morpho-agronomic characters have been used as a traditional method to identify cultivars and to select superior genotypes. Table (1) illustrated the morphological and agronomic characteristics of the three studied sweet potato genotypes; Abees, Mabrouka and Gendawy. Based on the measured data, Abees showed the highest values of stem length (195.0 cm) and No. of branches/plant (23 branches), while Mabrouka recorded the highest value of No. of leaves/plant (370 leaves), compared to the other studied genotypes. On the other hand, the genotype Gendawy recorded the lowest values for each of the three above traits. Regarding No. of root/plant, root length and diameter, yield/plant and average root weight, Gendawy gave the highest values among the studied genotypes.

These results indicated that Gendawy genotype recorded the highest values for all yield related traits. On contrast, this genotype did not exhibit any superiority in the morphological characters compared to Abees and Mabrouka cultivars. These potential variations among sweet potato genotypes may due to their genetic makeup.

With regard to the observations on stem color, the recorded data revealed that all the three genotypes had the same color of stem which was green. For leaf shape, it differed among the three genotypes. Leaf shape was deblylobed in Abees, lobed in Mabrouka and heart in Gendawy.

Examination of storage roots revealed that all the three genotypes; Abees, Mabrouka and Gendawy, have the same shape and skin color. They had elliptic shape and dark purple skin color. Moreover, the recorded data showed that the color of flesh root differed among the studied genotypes. It was orange in Abees, white in Mabrouka and dark orange in Gendawy.

The variations in morphological and agronomic characters of sweet potato were reported previously by Das and Naskar (2008) who used agronomical analysis for determining the genetic diversity among ten varieties of sweet potato. Also, Tairo *et al.* (2008) used morphological and agronomic traits in providing a preliminary characterization of the available sweet potato germplasm in Tanzania. Similar studies were performed with the sweet potato collections from Brazil (Veasey *et al.*, 2007), Uganda (Yada *et al.*, 2010), Tanzania (Elameen *et al.*, 2011) and South Africa (Laurie *et al.*, 2013).

#### Chemical characterization

The chemical composition of the three studied sweet potato genotypes is

presented in Table (2). It revealed that the highest moisture content was found as 42.00% in Abees cultivar, while the lowest value was recorded for Gendawy genotype (26.50%). On the other hand, Gendawy had the highest values of carbohydrate, carotene and protein contents (68.30%, 2.40% and 13.20%, respectively) compared to the other two genotypes; Abees and Mabrouka, which did not differ significantly. For Vitamin-C content, all the three genotypes did not differ significantly from each other and they recorded values between 0.11% for Abees and 0.57% for Mabrouka.

In agreement with the agronomic traits, results of nutritional characters confirmed the superiority of Gendawy over the other genotypes; Abees and Mabrouka. Gendawy recorded the lowest value of moisture content indicating that it had the highest values of dry matter, besides, the highest contents of carbohydrate, carotene and protein. The percentage of moisture in powder plays a very important role in the flow and other mechanical properties of the food. Thus, increasing dry matter content is the major objective in sweet potato breeding program. In general, Gendawy can be considered as the most significant genotype than the other two cultivars; Abees and Mabrouka, with regard to nutritional value.

Senanayake *et al.* (2013) analyzed the nutritional quality for five different cultivars of sweet potato commonly available in Sri Lanka. Their results showed that orange fleshed cultivars were comparatively rich in nutrients and digestibility than the other tested cultivars. Also, Sanoussi *et al.* (2016) compared ten selected local varieties in Benin Republic for their macro-nutritional composition. The results indicated that the orange flesh sweet potato cultivars recorded the highest nutrient content for most of the parameters studied; dry matter, protein, fiber and ash content.

# Molecular characterization based on RAPD markers

# a. Polymorphism

Nine RAPD primers were used to estimate the levels of polymorphisms and genetic relationship among the three sweet potato genotypes used in this study (Fig. 1). A total of 146 discrete amplified bands were generated from the nine primers with an average of 16.22 bands per primer (Table 3). Sixty nine bands were common for all genotypes (monomorphic), however, 77 bands were polymorphic with 52.74% polymorphism indicating a high level of polymorphism among the studied genotypes. Generally, the levels of polymorphism among the different sweet potato genotypes varied with the different primers. The highest number of polymorphic bands (19 bands) was amplified with primer OPB-01 while the lowest number (two bands) was detected for primer OPB-07. The size of the amplified bands also varied by using different primers, it ranged from 192 bp for primer OPB-10 to 2145 bp for primer OPB-01.

These results indicated that RAPD primers clearly determined the genetic

variability in the studied sweet potato genotypes. Similar results were reported by Gichuki et al. (2003) who used RAPD markers to study the genetic polymorphism among 74 accessions of sweet potato from different agroclimatic zones and found 71 polymorphic markers. This was also in agreement with that of He et al. (2006) who found high level of genetic diversity (218 polymorphic markers) in sweet potato accessions using 30 RAPD primers. A study of Moulin et al. (2012) on 59 samples of sweet potato collected from rural properties and 19 from local markets in Brazil; using 18 RAPD primers, revealed 145 polymorphic bands out of 150 amplified ones. Maquia et al. (2013) reported high levels of polymorphism (94.6%) in the sweet potato germplasm collected from Mozambique. Also, da Silva et al. (2014) used nine RAPD primers to analyze 52 genotypes of sweet potato from the North east of Brazil. They found a total of 50 bands with 100% polymorphism.

Based on these results, sweet potato exhibited a high level of genetic variation indicating that RAPD is a very effective method in DNA fingerprinting to establish genetic polymorphism and to determine the genetic variability among sweet potato genotypes.

### b. Genetic similarity and cluster analysis

Data of RAPD-PCR were also used to estimate the genetic similarity and the phylogenetic relationship among sweet potato genotypes. A similarity coefficient matrix among the three genotypes was presented in Table (4). Values of genetic similarity were 0.602, 0.718 and 0.540 between (Abees and Mabrouka), (Abees and Gendawy) and (Mabrouka and Gendawy), respectively, with an average of 0.620. This value was close to the value of 0.588 that was found among examined accessions from South America (Zhang et al., 1999) and the value of 0.691 that found among sweet potato cultivars in Taiwan (Tseng et al., 2002). Also, it was not different from the value of 0.709 that was found among Tanzanian sweet potato accessions (Elameen et al., 2008). Generally, the genetic similarity estimates varied depending on genotypes. Maquia et al. (2013) studied a collection of sweet potato germplasms from Mozambique and found genetic similarity with mean values around 0.52. Using 40 RAPD primers, Mohamed et al. (2016) observed genetic similarity values ranged from 0.655 (between Abees and accession No. 199035.7) to 0.939 (between accessions No. 199015.14 and No. 199026.1) with an average of 0.797.

The UPGMA dendrogram; based on the similarity index, assembled Abees and Gendawy genotypes into the same group, while Mabrouka cultivar was the most divergent (Fig. 2). Many studies assessed the degree of genetic relatedness among sweet potato genotypes based on RAPD markers. Gichuki *et al.* (2003) grouped several sweet potato accessions together based on their geographic origin suggesting an evolutionary relationship among them. He *et al.* (2006) studied genetic relationships among Chinese sweet potato landraces and found a moderate mean genetic distance of 0.58. In contrast, low diversity was observed among the sweet potato genotypes from the United States (He *et al.*, 1995) and from Papau New Guinea (Zhang *et al.*, 1998).

Although the close relationship between Abees and Gendawy genotypes at the molecular level (similarity value of 0.718), there were great variations among them for morpho-agronomic characters and the chemical composition. These results indicated that RAPD analysis could not be effective in separating the three Egyptian genotypes; Abees, Mabrouka and Gendawy, according to their morpho-agronomic or chemical characters. Thus RAPD analysis needs to combine with other specific molecular markers as well as the traditional characterization for a comprehensive consideration. This was in agreement with Huang and Sun (2000), they pointed to the role of molecular markers in sweet potato germplasm characterization indicating that molecular analysis was more efficient than morphological analysis in differentiating sweet potato cultivars. Tairo et al. (2008) reported that the morphological traits were not sufficient to characterize sweet potato cultivars and it should be supported by DNA-based markers. Lin et al. (2009) used RAPD assay in evaluating the genetic variations and its relationships along with morphological characters in the sweet potato. Gepts (2006) and Khoury et al. (2010) stated that the standard characterization of any crop germplasm should be included conventional approaches (i.e. morphological and agronomic characters), combined with molecular markers.

The results obtained in this study concluded that the assessment of genetic variability should be performed using morpho-agronomic evaluation and nutritional characters, combined with RAPD molecular markers. RAPD markers were effective in separation and identification of the variability of sweet potato. Abees and Gendawy genotypes showed a close genetic relationship, while Mabrouka was the most distinct genotype, indicating high genetic variability for using in breeding programs. Gendawy genotype had the highest values for most of yield and nutritional traits. Thereby, much attention should be paid to use this genotype as a parent in genetic improvement of sweet potato to get a new cultivar with high yield and good quality; which considered an important target for increasing the economic value of sweet potato.

# SUMMARY

Morpho-agronomic and chemical analysis as well as RAPD markers were used to determine the genetic diversity among three Egyptian genotypes of sweet potato; Abees, Mabrouka and Gendawy. The results revealed that there is a wide variation among the three genotypes in most morphological and agronomic characters in addition to the nutritional values. Gendawy genotype had the highest values for most agronomic and chemical traits compared to the other two genotypes; therefore it is considered a good source of agronomic and nutritional traits for breeding. Regarding molecular characterization, a total of nine RAPD primers were used to assess the genetic variability and relationships among the three sweet potato genotypes. A total of 146 amplified bands were generated from the nine primers with 52.74% polymorphism indicating high genetic variability. Cluster analysis revealed a close genetic relationship between Abees and Gendawy genotypes (similarity value of 0.718), while Mabrouka was the most distinct genotype. Results concluded that RAPD analysis could not be effective in separating genotypes according to their morphological, agronomic or chemical characters. In addition, characterization based on these conventional characters should be complemented with DNA-based molecular characterization to reveal genetic diversity in the three Egyptian sweet potato genotypes.

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Traits	Abees	Mabrouka	Gendawy
Stem length (cm)	195.0 <sup>a</sup>	185.0 <sup>b</sup>	95.0 °
No. of branches/plant	23.00 <sup>a</sup>	18.50 <sup>b</sup>	14.33 <sup>c</sup>
No. of leaves/plant	197.0 <sup>b</sup>	370.0 <sup> a</sup>	135.0 <sup>c</sup>
No. of root/plant	3.80 <sup>b</sup>	3.33 °	4.30 <sup>a</sup>
Root length (cm)	11.00 <sup>b</sup>	10.83 <sup>b</sup>	19.00 <sup>a</sup>
Root diameter (cm)	3.77 °	3.90 <sup>b</sup>	5.80 <sup>a</sup>
Yield/plant (g)	420.0 °	535.0 <sup>b</sup>	850.0 <sup>ª</sup>
Average root weight (g)	116.0 <sup>c</sup>	197.0 <sup>b</sup>	277.0 <sup>ª</sup>
Stem color	Green	Green	Green
Leaf shape	Deblylobed	Lobed	Heart
Root shape	Elliptic	Elliptic	Elliptic
Root skin color	Dark purple	Dark purple	Dark purple
Flesh root color	Orange	White	Dark orange

Table (1): Morphological and ag	gronomic characteristics	of the three	Egyptian sweet potato
genotypes.			

Means within the same row followed by different letters are statistically different at 5 % level.

Chemical composition (%)	Abees	Mabrouka	Gendawy
Moisture	42.00 <sup>a</sup>	38.50 <sup>b</sup>	26.50 <sup>c</sup>
Carbohydrate	48.00 <sup>b</sup>	51.00 <sup>b</sup>	68.30 <sup>a</sup>
Carotene	1.50 <sup>b</sup>	$1.60^{b}$	$2.40^{a}$
Vitamin-C	0.11 <sup>a</sup>	$0.57^{a}$	0.25 <sup>a</sup>
Protein	7.07 <sup>b</sup>	6.87 <sup>b</sup>	13.20 <sup>a</sup>

Means within the same row followed by different letters are statistically different at 5% level.

Table (3): RAPD primers and their oligonucleotide sequences, total number of bands and percentage of polymorphism among the three Egyptian sweet potato genotypes; Abees, Mabrouka and Gendawy.

Primer name	Sequence $(5^{\circ} \rightarrow 3^{\circ})$	Size of bands (bp)	Total amplified fragments	Monomor- phic bands	Polymor- phic bands	Polymor- phism (%)
OPA-09	GGGTAACGCC	213-2125	14	8	6	42.86
OPA-14	TCTGTGCTGG	480-1680	14	9	5	35.71
OPB-01	GTTTCGCTCC	350-2145	21	2	19	90.48
OPB-06	TGCTCTGCCC	274-1692	12	8	4	33.33
OPB-07	GGTGACGCAG	288-1333	11	9	2	18.18
OPB-08	GTCCACACGG	250-1622	15	5	10	66.67
OPB-10	CTGCTGGGAC	192-2058	25	13	12	48.00
OPB-11	GTAGACCCGT	238-1635	14	3	11	78.57
OPB-12	CCTTGACGCA	236-1718	20	12	8	40.00
	Total		146	69	77	52.74

Table (4): Genetic similarity matrix among the three Egyptian sweet potato genotypes based on RAPD markers.

	Abees	Mabrouka
Mabrouka	0.602	
Gendawy	0.718	0.540

GENETIC CHARACHTERIZATION OF THREE EGYPTIAN SWEET POTATO GENOTYPES

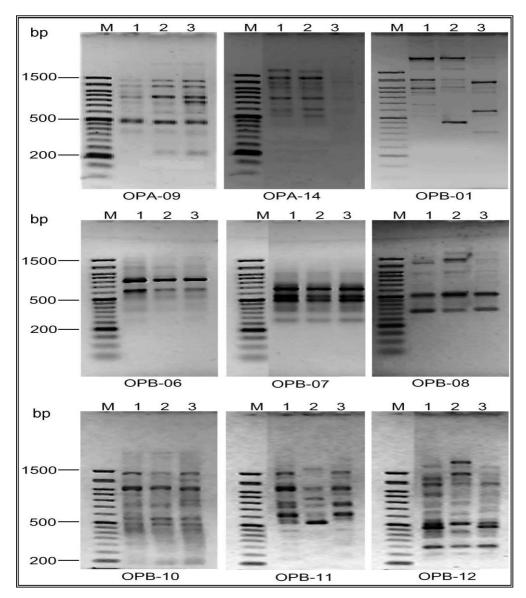


Fig. (1): RAPD profiles of the three Egyptian sweet potato genotypes. M: 50 bp DNA leader, 1: Abees, 2: Mabrouka and 3: Gendawy.

Fig. (2): UPGMA phylogenic relationship among the three Egyptian sweet potato genotypes based on RAPD markers.

