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Evaluation and Genetic Diversity of Three Selected White Sapote (*Casimiroa edulis*) Clones under Semi-Arid Climate

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Abstract: Three selected white sapote (*Casimiroa edulis*) clones (El-1, El-2 and El-3) derived from seedlings were grown under Sohag condition during 2009-2011 seasons at El-Kawther region and were evaluated for fruiting characteristics and their genetic diversity via SDS-PAGE. The full bloom, harvesting date and number of fruiting days (from full bloom to harvest) was earliest in El-3 clone and the latest in El-1. The highest average fruit yield/tree occurred in El-1 clone followed by El-2, then E1-3. All the studied clones were characterized by alternate bearing. As for fruit quality (fruit weight, flesh / fruit weight, seed's weight, number of seeds / fruit, TSS, acidity, TSS / Acid ratio, protein and fatty content), the highest values were found in El-1 clone, while the lowest occurred in El-2. Genetic diversity, viz., the number of polypeptides was 32 with their molecular weights ranging between 150 and 8 KDa. The SDS-PAGE protein patterns of the three clones showed distinct changes in eight protein bands. Generally El-1 genotype surpassed the other white sapote clones in the fruit yield and quality and had the latest harvesting date.

Key words: Casimiroa edulis · Fruit quality · Full bloom · Genetic diversity · White sapote and yield

INTRODUCTION

The white sapote (*Casimiroa edulis*) is an evergreen fruit tree that originated in the highlands of Mexico and Central America [1, 2]. White sapote readily adapts to subtropical climate, such as in the United States and Mexico in North America and in Egypt, where in addition to its fresh consumption [3], it is known to possess medicinal properties. Sapote cultivars display distinct differences in flowering, fruit set, fruit yield and as well as other fruit characteristics, i.e., the size, shape, skin and flesh color, brix levels and seed/flesh ratios [4-8].

In Egypt, all the white sapote trees are derived from seeds, resulting in great genetic and fruiting variation amongst trees. Selection superior clones from outstanding seedling progeny from these seedling fruit trees are considered one of the most important methods for improving Sapote cultivars. However, the parentage of many cultivars in unknown [9] and their genetic relationships remain unclear. Recently, SDS-PAGE is a practical and reliable method for species identification [10-12] and very useful tool for studying genetic diversity rapidly of white sapote [7, 8].

This study was conducted to evaluate three new white sapote clones raised from seedlings and selected on the basis of fruit morphology and to identify the genetic diversity among them using the SDS-PAGE protein patterns in order to select the best one to be used in vegetative propagation for commercial production. The evaluation of white sapote clones was based on the variation between the full blooms, harvesting date, number of fruiting days (from full bloom to harvest), fruit yield, alternate bearing index and fruit quality and correlate them to their SDS-PAGE characteristics.

MATERIALS AND METHODS

The current study was carried out during the three successive seasons of 2009, 2010 and 2011 on three white sapote clones raised from seedlings, selected on the basis of fruit morphology and named 'El-Kawther1' (El-1), 'El-Kawther2' (El-2) and 'El-Kawther3' (El-3). The clones were grown at the Experimental Farm of Faculty of Agriculture, Sohag University, located at El-Kawther region, Sohag Governorate. The trees where about 7 years old at the time the study began. They were grown

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in sandy calcareous soil (containing 18.8% $CaCO_3$) 6 × 6 m apart, irrigated using flood system with specific management for fertilization, pruning and control of pests and diseases.

Experimental Design: The selected replicates of the white sapote clones were similarly in appearance and were employed in a complete randomized design (CRD) experiment. Each clone was represented by four trees in 4 replicates with one tree per replicate.

Experimental Work: The following parameters were evaluated in all the three selected white sapote clones:

- Full bloom (at 50 – 70% anthesis), this stage was estimated by examining several inflorescences around the tree canopy;
- Harvesting date;
- Number of days from full bloom to harvest;
- Fruit Yield / tree and alternate bearing index: Fruit yield as weight (kg) per tree was recorded for each clone at harvest time. The index of alternate bearing per individual trees estimated according to following equation suggest by [13]. Alternate bearing index = 100 × (difference between two successive yield / sum of two successive yield). If the result indicated more than 25% this means that, tree is alternate bearing habit. While the tree is regular bearing if the result was less than 25%;
- Fruit quality: Ten fruits per tree for each clone, was picked at harvest time (in 1 – 2 weeks the fruits will soften and reached full color stage) to determine the following measurements:

- Physical properties: Fruit weight (g), fruit shape, fruit color (Peel and Pulp), flesh / fruit weight percentage, seed's weight (g) and number of seeds / fruit (well developed and aborted); and Chemical properties: Total soluble solids (TSS) percentage in flesh juice was determined by using a hand refractometer, while total acidity percentage was estimated as malic acid, according to [14]. Flesh (0.5g) of dried sample was digested using the H_2SO_4 and H_2O_2 as described by [15], nitrogen content (g/100g D. wt) was determined in the digested solution by the modified micro-kjeldahl method as described by [16], crude protein content was calculated by multiply total nitrogen by 6.25 according to [17]. The lipids of a known weight of the flesh (5g) were extracted with petroleum ether for 18 hours in soxhlet apparatus. The solvent was evaporated and the residue was dried to a constant weight at 95°C according to [17], the percentage of crude lipid content (g/100g) was then calculated on a dry weight basis;

- Genetic diversity: Approximately 1g fresh plant materials (fresh leaves) for each clone were ground in a mortar and pistil in liquid nitrogen. Crashing continued until the plant materials were completely homogenized. The crashed samples were mixed with (1ml) extraction buffer (50 mM Tris – HCI buffer, Ph 6.8, glycerol 10% W/v), ascorbic acid 0.1% and cysteine hydrochloride (0.1% W/v), after centrifugation at 15000 rpm for 15 min, 10% SDS were added to the samples and heated to 96°C in a water path for 2-5 min. electrophoresis and commassie blue staining method for the SDS PAGE were performed using 11% polyacrylamide gel at 10°C and 60 mA according to [18].

Statistical Analysis: The obtained data were statistically analyzed using the MSTAT-C statistical analysis package by [19] and then the LSD test was used to recognize the significance between the treatment means according to the procedure of [20].

RESULTS AND DISCUSSION

Data in Table 1 shows that the full bloom, harvesting date and number of fruiting days between different white sapote clones and seasons varied considerably. Full bloom and harvesting dates were earliest in the El-3 clone and latest in El-1 clone regardless of the growing year. However, variations among the studied clones in the full bloom, harvesting date and number of fruiting days were evidently due to the variation in their genetically background and climatic conditions [21]. These results are similarly obtained in other white sapote tree studies [4, 6, 22].

Fruit yield (kg/tree) varied considerably amongst the different in the clones in the different growth seasons (Table 2). In the first season, El-1 had the highest fruit yield compared to the other clones followed by El-2 and El-3. In the second season, El-2 had the highest fruit yield followed by El-1 and El-3. In the third season, El-1 had the highest fruit yield followed by El-2 and El-3 clones (no significant differences between them for this season).

The average tree yield revealed that El-2 and El-1 clones were considerably higher in the second season compared to the first and third one while; El-3 clone was consistently the lowest. The highest average tree yield in seasons of study was recorded in El-1 clone followed by

Table 1: Full bloom, harvesting date and number of days from full bloom to harvest of three white sapote clones during 2009, 2010 and 2011 seasons.

	Full bloom			Harvesting date			Number of days (full bloom – harvest)		
Clones	2009	2010	2011	2009	2010	2011	2009	2010	2011
C_1	12 Mar.	9 Mar.	13 Mar.	30 July	25 July	3 Aug.	140	138	143
C_2	25 Feb.	17 Feb.	4 Mar.	15 June	4 June	24 June	111	108	112
C_3	12 Feb.	10 Feb.	21 Feb.	30 May	23 May	9 June	108	103	106

Table 2: Yield and average yield (kg)/tree of three white sapote clones during 2009, 2010 and 2011 seasons.

	Yield (kg) / tree			Average yield (kg) / tree between	
Clones	2009	2010	2011	2009 / 2010	2010 / 2011
C_1	59.90	29.93	50.00	44.92	39.97
C_2	20.00	34.93	14.60	27.47	24.77
C_3	14.97	20.00	11.63	17.49	15.82
LSD at 5%	0.72	0.12	3.69	-	-

Table 3: Alternate bearing value and fruit weight (g) of three white sapote clones during 2009, 2010 and 2011 seasons.

	Alternate Bearing Index between		Fruit weight (g)		
Clones	2009 / 2010	2010 / 2011	2009	2010	2011
C_1	33.37	25.11	119.90	133.77	130.37
C_2	27.18	41.05	134.47	127.47	131.80
C_3	14.39	26.47	59.23	53.10	57.93
LSD at 5%	-	-	27.55	29.17	13.74

Table 4: Fruit shape and fruit color of three white sapote clones during 2009, 2010 and 2011 seasons.

	Fruit shape			Fruit color	
Clones	2009	2010	2011	peel	pulp
C_1	1.09	1.21	1.25	Light green	Creamy
C_2	1.06	0.93	0.91	Yellow	Yellow
C_3	1.03	1.18	1.20	Yellow	Yellow
LSD at 5%	0.10	0.23	0.18	-	-

Table 5: Flesh/fruit weight % and seed's weight (g) of three white sapote clones during 2009, 2010 and 2011 seasons.

	Flesh/fruit weight %			Seed's weight (g)		
Clones	2009	2010	2011	2099	2010	2011
C_1	93.07	93.33	92.90	8.40	8.73	9.23
C_2	84.77	84.03	84.97	20.50	20.33	19.67
C_3	87.60	87.70	85.93	7.27	6.10	8.10
LSD at 5%	4.67	10.28	4.64	2.53	6.25	3.34

Table 6: Number of seed/fruit of three white sapote clones during 2009,2010 and 2011 seasons.

	Number of seed's / fruit					
	Well developed			Aborted		
Clones	2009	2010	2011	2009	2010	2011
C_1	2.00	1.33	1.67	3.00	3.67	3.33
C_2	4.00	3.67	4.00	1.00	1.33	1.00
C_3	2.33	2.00	1.67	2.67	3.00	3.33
LSD at 5%	1.50	0.93	0.75	1.50	0.93	0.76

Fig. 1: Picture of white sapote clones fruit

Table 7: Total Soluble Solid (TSS), acidity% and TSS/acid ratio of three sapote clones during 2009, 2010 and 2011 seasons.

	TSS%			Acidity%			TSS/acid Ratio		
clones	2009	2010	2011	2009	2010	2011	2009	2010	2011
C_1	15.00	17.40	15.27	0.19	0.19	0.18	79.17	93.33	83.53
C_2	14.33	16.53	14.40	0.22	0.22	0.21	65.17	76.40	66.57
C_3	14.73	17.33	17.87	0.20	0.20	0.20	72.40	86.90	73.23
LSD at 5%	2.55	0.14	0.68	0.02	0.02	0.02	12.77	3.57	6.43

Table 8: Protein and fatty percentage of three white sapote clones during 2009, 2010 and 2011 seasons.

	Protein %			Fatty %		
Clones	2009	2010	2011	2009	2010	2011
C_1	0.97	0.93	0.97	0.73	0.73	0.77
C_2	0.67	0.77	0.67	0.50	0.50	0.53
C_3	0.70	0.77	0.67	0.53	0.50	0.53
LSD at 5%	0.07	0.07	0.12	0.07	0.07	0.14

El-2 and El-3 respectively. We attribute these fruiting yield variations of different clones due to their cultivar genetic properties [4, 6].

The alternate bearing index in white sapote clones shown in Table 3 expresses the [13] equation. All studied white sapote clones between 2010 and 2011 seasons were characterized by alternate bearing because the index was higher than 25% (Table 3). In 2009 and 2010 seasons, El-3 clone was characterized by regular bearing because the alternate bearing index was less than 25% (14.39). The alternate bearing habit seems to be mainly related to some inheritance factors and partially to the environmental factors [21].

The physical properties of the fruits are expressed in Fig. 1 and Tables 3, 4, 5 and 6. The fruit weight generally ranged from 53.10 to 134.47 (g) at harvest time and the least value (53.10) was recorded in the El-3 clone followed by El-1 (133.77) and then El-2 (134.47) with no signification differences between them.

Fruit shape varied among white sapote clones (Table 4). The fruit peel color ranged from light green in El-1 clone to yellow in El-2 and El-3 (Table 4). The pulp color of El-1 clone was creamy while it was yellow in El-2 and El-3 (Table 4)

Flesh per fruit weight percentage indicated that flesh per fruit percentage in El-1 was significantly higher than the other white sapote clones (Table 5). The seed's weight for El-2 fruits was significantly larger when compared to the other clones (Table 5).

Number of fertile seeds per fruit and number of aborted seeds per fruit varied among the three clones (Table 6). Similar results have been obtained by other researchers [4-6, 22].

El-1 fruits have significantly higher TSS accompanied with lower percentage of acidity than the other clones which in turn reflected on TSS/acid ratio which was significantly higher in El-1 fruits followed by El-3 then El-2 clones in this respect (Table 7).

Depending on the clone, protein content ranged from (0.67 to 0.97%) and fatty acids (0.50 to 0.77%) at ripening stage. The highest value of protein (0.97) and fatty acids (0.77) were recorded in El-1 clone while the least values (0.67) and (0.50) respectively were recorded in El-2 and El-3 with non-significant differences between them.

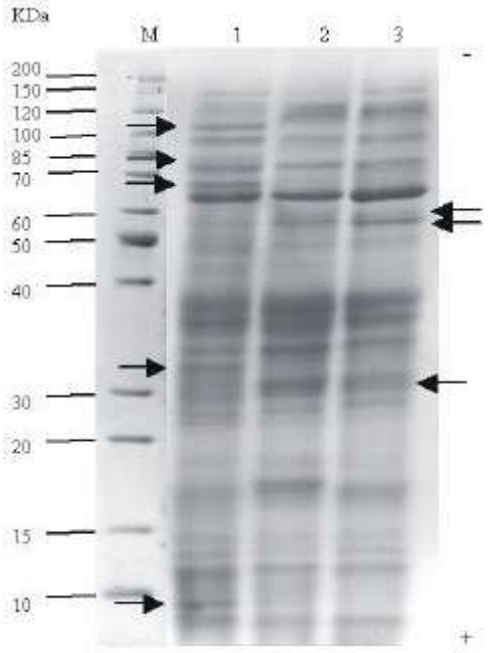

Fig. 1: Protein profile on SDS-PAGE of different white sapote clones. Clone 1 (lane 2), clone 2 (lane 3), and clone 3 (lane 4). Lane 1 represents protein marker.

These results correlate with those reported by other investigators [4, 5, 6]. In view of the statistical analysis of fruit physical and chemical properties, it was observed that the highest values were recorded in El-1 clone while the lowest values were recorded in El-2 and El-3.

Genetic Diversity: The protein SDS-PAGE of the three white sapote clones genotypes was carried out to investigate their genetic diversity. The number of polypeptides obtained in these clones was 32 with their molecular weights ranging between 8 to 150 KDa. Bands were classified into distinct three regions depending on molecular weight: high molecular weight (X region), intermediate molecular weight (Y region) and low molecular weight (Z region). The SDS-PAGE protein patterns of the three white sapote clones showed changes in the eight protein bands (Fig. 2). Five protein bands were detected in the X region. Three of these had molecular weights of 65, 83 and 105 KDa occurred in first clone (El-1) and were not present in the other two clones. Two bands in the same region had molecular weights of 56 and 60 KDa; these were detected in the second and the third clone (El-2 and El-3), but not detected in the first clone. Also, one protein band with a molecular weight of 34 KDa was present in the first clone but was not present in the two other clones in the region Y and one band in the same region with molecular weight of 32 KDa was found in the second and the third clones, but not detected in the first one. Finally, only one protein band from the eight different bands with molecular weight of 9 KDa was detected in the third region Z, was found only in the first clone and disappeared in the two other clones.

Genetic variability in plant cultivars on the basis of protein and isoenzyme polymorphism was reported by [7, 8, 10-12]. In the current work, the suitability of protein studies in the field of evaluation of genetic variation in the tested clones was detected.

From the aforementioned results one can conclude that El-1genotype surpassed the other white sapote clones in the fruit yield and quality and had the latest harvesting date. In addition, the SDS-PAGE protein patterns of the three clones showed distinct changes in eight protein bands.

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