Evaluation of three genotypes of *Swietenia mahagoni* trees

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ABSTRACT

The use of mahagoni timber began long time ago from the eighteen century in the Caribean islands. It was first used as a substitute for the oak timber for shipbuilding. However, this tree is now endangered. This study highlighted the new super mahagoni tree that is tolerant unfavorable environment and which is the only survived one from one hundred trees that were cultivated in Nobaria location. This study was designed to screen morphological attributes and describe similarity and diversity in terms of isozyme, RAPD and ISSR profiles of three genotypes of Swietenia mahagoni and to compare them and investigate a new genetic resource (Nobaria location). Three different genotypes of Swietenia mahagoni were obtained from Alexandria governorate, timber tree department orchard of Horticultural Institute Research, Giza governorate and North Tahreer region, Beheira province. Soil characterization was done. Antioxidant compounds were extracted and determined for the samples. Isoenzyme electrophoresis was done. Characterization of three different genotypes of Swietenia mahagoni at molecular level was done. Also, sequence repeat was done. Results revealed that calcium carbonate contents of the samples were found to be high which causes the fixation of the phosphorous. Total phenolic compounds, flavonoid content and antioxidant activity were found to be of a significant difference among the three genotypes. The highest flavonoids content was observed in the leaves of Alexandria tree genotype. Our results indicated that trees grown in salinity (Nobaria tree genotype) conditions had the highest amount of total phenolics, total flavonoids and total antioxidant compared to trees grown in non-saline conditions. The results found that Nobaria genotype bands had higher densities and intensities than in the other two locations in peroxidase and polyphenol oxidase banding pattern. At the molecular level, four RAPD-primers displayed a total of 42 DNA fragments were detected, in which 26 (61.90%) were polymorphic fragments. However, 16 bands were common (monomorphic) for the three genotypes. In ISSR analysis, 6 of the ISSR primers generated variable banding patterns. A total of 39 out of 62 ISSR fragments were polymorphic. 28 amplified fragments were considered as cultivar-specific markers. Results of the combination of the banding patterns of both techniques, wood chemistry revealed that high level of variation was on Nobaria mahagoni tree station which recorded high extracts percentage against Giza that recorded the lowest percentage. Hemicelllose analysis showed that the tree of Nobaria recorded the highest percentage compared to corresponding trees in Alex. and Giza. Fiber length analysis showed that Nobaria tree recorded the tallest fiber length against the two other locations. In conclusion, similarities and differences were identified between the three genotypes and proved that pattern resistant genotype of Swietenia mahagoni, cultivated at Nobaria, Horticultural Research Station, Egypt showed good tolerance to unfavorable environmental conditions (salinity and calcareous soil).

***Key words**: Mahagoni timber, Isozyme, Genotypes, Antioxidants, Polyphenols, Molecular markers, Anatomical study, Wood chemistry, Wood extract, Hemicellulose, Fiber length.*

INTRODUCTION

The history of international trade in mahagoni timber started in the late eighteenth century with the exploitation of small-leaf (*Swietenia mahagoni*) mahagoni, from the Caribbean islands, for European markets. It was used as a substitute for oak in shipbuilding and for walnut in furniture making when these woods were becoming difficult to obtain (Keay, 1996). The trade established the high value and high reputation of mahagoni timber that continues today. Small-leaf mahagoni was so heavily exploited that it had apparently become extinct in commercial sizes in some places. *Swietenia mahagoni* Jacq belongs to Family *Meliaceae,* it is semi-ever green trees and considered tropical forest in west India (Chudnoff, 1984). Moreover, *Swietenia mahagoni* products are fuel, in Haiti much of the branch wood and most of the crooked stems are converted to charcoal, particularly in regions isolated from urban markets by poor roads. The heartwood is highly resistant to decay and insect attack, performing better than all other mahagonies on the world markets. The wood is therefore the choice for high-quality furniture and cabinet work joinery, boats and pattern work. *Swietenia mahagony* seed is flat and dark-brown in colour. Mahagoni has been planted as windbreak or near housing as offices canopy tree. For resident, mahagoni's seed was used for reducing blood pressure, anti-fungal, anti-fever, reducing glucose levels, and anti-rheumatic. The bark of mahagoni can be used for reducing fever and astringent (Harianja, 2008). This tree soundly gave a lot of benefits in reducing many diseases. However, a little is known about the using of mahagoni (*S. mahagoni*) seed or other parts of this tree for reducing malaria infection (Upiek *et.al*., 2013).

Study sites description

Nobaria represented in Figure (1) is research station, which located in markaz Abu Al Matamir, El- Baheira governorate (30.91 Nº, and 29.97 E º). The climate is semiarid mediterranean, characterized by an intense summer drought and a mean annual rainfall of 320 mm. Mean annual temperature is 15.0 Cº, with a mean minimum temperature in the coldest and a mean maximum temperature in the hottest. Antoniades (Fig.2) garden followed qusm Sidi Gabir at Alexandria governorate (31,20 Nº, 29.95 Eº). The climate is semiarid mediterranean. Fig. (3) showed location of Horticultural Research Institute, Agriculture research center, Giza governorate (30.02 Nº, 31.21 Eº), the climate is drought worm in winter and hot in summer.

Fig. (1) Nobaria.

Fig. (2) Alexandria.

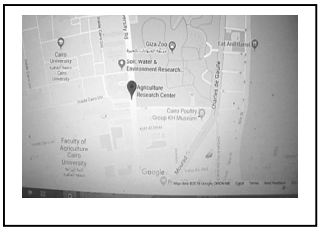


Fig.(3) Giza.

Genetic diversity is the basis for adaptability and is essential for long term stability of populations and tree breeding for production, whether in plantations or by natural regeneration. It provides the potential for species to resist pests and diseases, and adapt to different environments. The conservation of genetic diversity and genetic resources is particularly recognized by the Intergovernmental Panel on Climate Change (IPCC, 2007) as important for adaptation to predict climate change. Deforestation reduces the genetic diversity of trees through direct loss of diversity, disruption of gene flow, and genetic isolation (Lowe *et al.,* 2005), which can lead to inbreeding and associated reductions in fitness (Bawa and Dayanandan, 1998). But within certain limits, gene flow and resilience allows forest trees to adapt to fragmentation (Hamrick, 2004). Added to these impacts, economically important species are faced with additional pressures, such as selective removal of phenotypically superior individuals during logging (possible dysgenic selection) and consequent devaluation of the remaining stand (Navarro and Hernandez 2004). The contrasting interests of production and ecological restoration mirror underlying scientific issues. The source of planting stock needs to be considered at both the population level and the individual level, selection interact to influence population delimitation and reproductive fitness. The relationship between genetic diversity, habitat heterogeneity, and the scale of adaptation in trees is complex and involves a variety of factors. Gene flow may counteract even fairly strong selection and prevent the formation of locally adapted populations, although very strong environmental variation (hence selection pressure) may produce adaptive differences over short distances, despite continued high levels of gene flow.

Since the genetic composition of seed is affected by patterns of pollen flow, the extent of localized adaptation and fitness may vary with pollen flow from differing environments. Secondary metabolites are phytochemicals produced as by-products of primary metabolism (Bako and Aguh, 2007) and are less widespread in plants. It is of course this restricted occurrence among plants that renders them valuable and useful in taxonomic delimitation of species. Random Amplified Polymorphism DNA (RAPD) markers are a modification of Polymerase Chain Reaction (PCR) used in the late 1980 (Williams *et al*., 1990). Among PCR based molecular markers RAPD is a widely used technique in different plants (Nazar and Mahmood, 2011 and Mahmood *et al*., 2010 a and 2011 b). PCR technique is one of the best available DNA-based tools for scoring variations between cultivars within species (Lakshmikumaran and Bhatia, 1998). In the present study, screening morphological attributes was done. Describing the similarity and diversity in terms of isozyme, RAPD and ISSR profiles of three genotypes of *Swietenia mahagoni* was also done.

MATERIALS AND METHODS

This study was carried out at Biotechnology Research Laboratory, Central Laboratory and Timber Trees and Foresting Research Department, Horticultural Research Institute, ARC, Egypt during the period of 2014- 2018, on three different genotypes of *Swietenia mahagoni*. One genotype grown at Alexandria governorate and the second one grown at the orchard of Horticultural Research Institute, Giza governorate, and the third genotype grown at Nobaria, Horticultural Research Station, North Tahreer region, Beheira province. Selected trees were about 30 years old.

Soil characteristics

Soil samples were collected from each of the studied orchards at depth of 30 to 60 cm and analyzed for Physico-chemical characteristics according to Wilde *et al.,* (1979) as shown in Table (1).

Table (1): Physico-chemical characteristics of the investigated Research Stations orchard loamy sand soil.

The physical and chemical properties of the soil.

Samples	pH	EC ds/m	Cations (meq/l)				Anions (meq/l)		
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_4^{--}
1	7.96	7.8	19.4	11.6	43.5	0.5	1.2	1.2	4.3
2	8.10	3.53	13.22	11.20	13.91	4.49	7.23	11.90	17.15
3	7.99	11.7	24.7	13.7	56.3	0.13	1.5	105	3.5

	Particle size distribution (%)		
	Clay	Silt	Sand
1	5	9.5	85.5
2	54.93	16.78	28.29
3	8.9	18.6	72.5

1, 2 and 3 = soil samples of Alexandria, orchard of Horticultural Research Institute, Giza governorate and Nobaria Horticultural Research Station, Beheira province respectively. EC = Electrical conductivity, PH = Acidity algorithm, ds/m = descisiemen/meters.

Sample preparation and extraction for some Antioxidant compounds

For methanolic extraction: grounding (2 g) green leaves in a pestle with 20 ml of 80% methanol. The homogenate was filtered to obtain methanolic extraction colorless.

Spectrophotometric measurements

The spectrophotometric measurements were performed using an ultraviolet-visible spectrophotometer (model MA9523-SPEKOL 211, ISKRA, Horjul, Slovenia).

Total phenols

The total phenolic contents of methanolic extract were determined according to (Singleton *et al*., 1999) by folin-ciocalteu reagent. The absorbance was recorded at 725nm.

Total flavonoids

Total flavonoids were estimated using method of (Woisky and Salation, 1998**)** using aluminum chloride; the absorbance was measured at 420 nm.

Total antioxidant capacity

The total antioxidant capacity of three different genotypes of *Swietenia mahagoni* leaves extracts was evaluated by the phosphomolybdenum method by Prieto *et al*., (1999). The absorbance of the solution was measured at 695 nm with a spectrophotometer against methanol as the blank. Ascorbic acid (AA) was used as the standard.

Isozymes electrophoresis

Extraction of three different genotypes of *Swietenia mahagoni* leaves isozymes was used as described by Jonathan and Weeden, (1990).Native– polacrylamide gel electrophoresis (Native-PAGE) was

performed in 12% (W/V) slab gel (Davis 1964). The gel was stained after run according to Tanksely and Rick (1980) for Poly Phenyl Oxidase (PPO) isozymes and Graham *et al*. (1964) for peroxidase isozymes. The staining gel was incubated at 37 °C in dark for complete staining after adding the appropriate substrates and staining solutions.

Gel documentation

Gels were photographed scanned, analyzed using Gel Doc Vilber Lourmat system to capture the image and to calculate band intensities.

Characterization of three different genotypes of *Swietenia mahagoni* at Molecular Level

Random Amplified Fragment DNA (RAPD-PCR) Analysis

In order to obtain clear reproducible amplification products, different preliminary experiments were carried out in which a number of factors were optimized. These factors included PCR temperature cycle profile and concentration of each of the template DNA, primer, $MgCl_2$ and Taq polymerase. A total of twenty random DNA oligonucleotide primers were independently used according to Williams *et al.* (1990) in the PCR reaction. Only five primers succeeded to generate reproducible polymorphic DNA products. The PCR amplification was performed in a 25 µl reaction volume containing the following: 2.5 µl of dNTPs (2.5 mM), 1.5µl of Mg Cl_2 (25 mM), 2.5 µl of 10x buffer, 2.0 µl of primer (2.5 µM), 2.0 µl of template DNA (50 ng/µl), 0.3 µl of Taq polymerase (5 U/µl) and 14.7 µl of sterile ddH2O. The reaction mixtures were overlaid with a drop of light mineral oil per sample. Amplification carried out in Techni TC-512 PCR System. The reaction was subjected to one cycle at 95 ºC for 5 minutes, followed by 35 cycles at 96 ºC for 30 seconds, 37 ºC for 30 seconds, and 72 ºC for 30 seconds, then a final cycle of 72 ºC for 5 minutes. PCR products were run at 100 V for one hour on 1.5 % agarose gels to detect polymorphism between the three different genotypes of *Swietenia mahagoni* under study. Only five primers succeeded to generate reproducible polymorphic DNA products. Table (2) lists the base sequences of these DNA primers that produced informative polymorphic bands. The PCR products were separated on a 1.5 % agarose gels and fragments sizes were estimated with the 100bp ladder marker (1500, 1000, 900, 800, 700, 600, 500, 400,300,200 and 100 bp).

Inter simple sequence repeat (ISSR-PCR) analysis

ISSR-PCR reactions were conducted by using six primers. Amplification was conducted in 25 µl reaction volume containing the following reagents: 2.5 µl of dNTPs (2.5 mM), 2.5 µl MgCl2 (2.5 mM), and 2.5 µl of 10 x buffer, 3.0 µl of Primer (10 pmol), 3.0 µl of template DNA (25 ng/µl), 1 µl of Taq polymerase (1U/ µl) and 12.5 µl of sterile dd H_2O. the PCRs were programmed for one cycle at 94º C for 4 min. followed by 45 cycles of 1 min. at 94 ºC, 1 min. at 57 ºC, and 2 min at 72 ºC the reaction was finally stored at 72 ºC for 10 min. The PCR products separated on a 1.5 % agarose gels and fragments sizes estimated with the 100bp ladder marker. (1500, 1000, 900, 800, 700, 600, 500, 400,300,200 and 100 bp). Only six primers succeeded to generate reproducible polymorphic DNA products. Table (2) lists the base sequences of these DNA primers that produced informative polymorphic bands.

Table (2): List of the primer names and their nucleotide sequences of RAPD and ISSR procedures used in the study.

ISSR Primer		RAPD Primer	
Primer Name	**Sequence**	**Primer Name**	**Sequence**
HB-8	5`GAG AGA GAG AGA GG 3`	OP-A07	5´GAAACGGGTG3´
HB-9	5`GTG TGT GTG TGT GC 3`	OP-A10	5´GTGATCGCAG3´
HB-10	5`GAG AGA GAG AGA CC 3`	OP-B07	5´GGTGACGCAG 3´
HB-11	5`GTG TGT GTG TGT TGT CC 3`	OP-B11	5`GTAGACCCGT 3`
HB-12	5`CAC CACCAC GC 3`	OP-C12	5´TGTCATCCCC 3´
HB-15	5`GTG GTGGTG GC 3`		

Anatomical study

Transverse cross sections were taken from the middle part of the main root (3-5 cm behind apex of the main root)at the method described by (Johansen, 1940). Samples were killed and fixed in formalin, acetic acid and ethyl alcohol, at a ratio of 90:5:5, dehydrated in ascending concentrations of ethyl alcohol, then cleared by soaking in a series of absolute alcohol and xylene and imbedded in paraffin wax (M.P. 55-58 °C). Using a rotary microtome, a serial cross-sections (15-20 microns) were taken. Samples were then stained with safraneen and light green combination and mounted in Canada balsam. Examination and observations were carried out by Nikon light Microscope and photographed by Nikon Camera FX-35. Customary methods of dehydration, infiltration and paraffin embedding were based on (Johansen, 1940).

Wood chemistry

Acid detergent fiber (ADF)

One liter of 1 N sulfuric acid was prepared and the normality was checked by titration, then 20 g of cetyl trimethyl ammonium bromide (CTAB) were added and stirred until dissolving. A known weight of ground sample (1 g) was mixed with 100 ml of acid-detergent solution. The mixture was boiled under reflux condenser for 60 min, then filtered through Gooch crucible. The residue was washed with hot distilled water, then with acetone and hexane, respectively, and dried at 100 °C for 8 h (or overnight) and weighed to calculate the acid-detergent fiber (A.O.A.C. 1995).

Hemicellulose

Hemicellulose was calculated by the difference between neutral detergent fiber and acid-detergent fiber.

Statistical analysis

Phytochemical data were subjected to analysis of variance according to Snedecor and Cochran (1980). Mean separation was done using least significant difference (LSD) test at 1 and 5% levels. All data represented as means of triplicate ± standard error. The DNA bands generated by each primer counted and their molecular sizes compared with those of the DNA markers. The bands scored from DNA profiles generated by each primer were pooled together. All data set performed by SPSS (version. 14.0) Program.

RESULTS AND DISCUSSION

Soil analysis

Data in Table 1 showed that, soil texture of Nobaria station is mainly calcareous sandy loam. The values of the EC are 11.7ds/m which is classified as very high saline soil while, CaCO3 values are 30.7, and the pH values are 7.99. The high contents of calcium

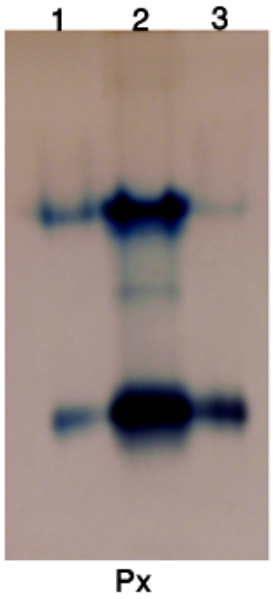
carbonates causes fixation of phosphorus, low availability of certain micronutrients (B, Fe, Zn, Ni, and Cu), and weak top soil structure (Anter *et al*., 1973).

Total phenolics, total flavonoids and total antioxidant contents

The present study was carried out on methanolic extracts of three genotypes of *Swietenia mahagoni* leaves to investigate the presence of medicinally important phytochemicals in the leaves**.** Data presented in Table (3) showed that, the leaves of all studied genotypes contain significant amount of phenols, flavonoids and antioxidant content. Phenols content was quantitatively estimated and it was (163.92, 165.05and 165.84mg/100g) for Giza, Nobaria and Alexandria tree genotype respectively. Flavonoids content of the three genotypes of *Swietenia mahagoni* leaves were in a descending order as the values were (4.24, 3.48 and 2.36mg/100g) where the locations of genotypes were Alexandria, Nobaria and Giza respectively Table (3). Similarly, all the genotypes exhibited good quantity of total antioxidant capacity, the maximum content (1161.41 mg/100g) was found in Nobaria tree genotype, 1146.06 mg/100g was in Giza tree genotype and the minimum content (1098.82mg/100g) was found in Alexandria tree genotype. Our results observed that trees grown in salinity (Nobaria) conditions had the highest amount of total phenolics, total flavonoids and total antioxidant compared to the plants grown in non-saline conditions, it is in line with Yuan *et al*. (2010) who stated that, phenolic compounds in plants are generated *via* the phenylpropanoid cycle and can be induced by abiotic stresses. Flavonoids have been linked to defense against various stresses (Winkel, 2002). Meanwhile, Selmar and Mohamed (2007) stated that, the content of secondary plant metabolites indeed is higher in plants that suffer from drought and salt stresses than those cultivated under optimal conditions.

Table (3): Some antioxidant contents for leaves of three genotypes of Swietenia mahagoni.

Samples	Phenols	Flavonoids	Total Antioxidant
	(mg/100g)	(mg/100g)	(mg/100g)
Alexandria tree	165.84 a	4.24 a	1098.82 c
Nobaria tree	165.05 b	3.48 b	1161.41 a
Giza tree	163.92 c	2.36 c	1146.06 b
L.S.D at 5%	0.34	0.12	7.1636



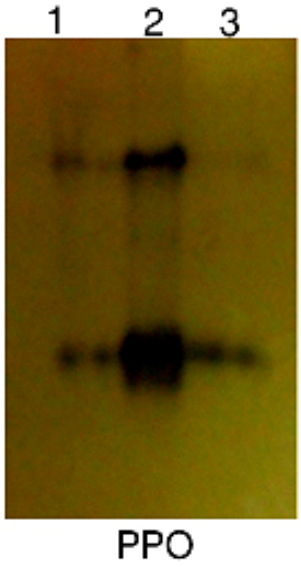


Fig.(4): Peroxidase isozymes bands identification of three genotypes of Swietenia mahagoni.

Fig.(5): Poly pahenyl oxidase isozymes bands identification of three genotypes of Swietenia mahagoni.

1= Alexandria tree genotype 2= Nobaria tree genotype 3= Giza tree genotype

Isozyme electrophoresis

Peroxidase banding patterns

Expression of the peroxidase enzyme was detected in leaf tissue extracted from the three genotypes of *Swietenia mahagoni* using 10% native PAGE (Fig.4). Fig.4 represent peroxidase electrophoresis banding patterns among examined leaves of *Swietenia mahagoni* samples, there were some differences in density of bands compared with each other and all three peroxidase bands (Px1, Px2 and Px3).

The results showed that, three bands were exhibited with different densities and intensities in three samples, these bands were approximately similar in Rf values and intensities, exceptionally, the Nobaria genotype bands were of higher densities and intensities than in the other two locations. Fig.4 explains that, the total of three bands were characterized for this study which were two of them (Px1 and PX3) was present in all treatments, but in high density in the Nobaria tree genotype and they could be considered as common bands. While, Px2 was present Nobaria tree genotype and absent in the two other locations which could be considered as positive specific marker. These results indicated that, salt stress increased the accumulation of peroxidase enzyme and that the encoding gene (s) was accelerated in response to salt stress. These results are in agreement with the finding of Sreenivasulu *et al.* (1999) who reported that, high peroxidase isozymic activity was found tolerant cultivar compared to salt susceptible cultivar of Fax-tail millet which related to the salt adaptation process. However, Rashed *et al.* (1994) reported that the occurrence of differential response in the decreases of intensity rather than in the isoforms of peroxidase in favor of

salt tolerant genotype under stress. Polyphenol oxidase electrophoretic patterns are illustrated in Fig. 5. Two bands with different intensities were observed among the profiles of the three genotypes of *Swietenia mahagoni*. One band was presented in all genotypes (monomorphic bands). Another band was presented in Alexandria tree genotype and Nobaria tree genotype and absent in Giza tree genotype (polymorphic band). These results are in agreement with El-Sayed *et al*. (2007) who found that, salinity and gamma rays caused the appearance and disappearance of bands in two wheat cultivars. Fig.5 represents poly phenyl oxidase electrophoresis banding patterns among examined leaves of the three genotypes of *Swietenia mahagoni* samples; there were some differences in density of band in all two poly phenyl oxidase bands (PPO1 and PPO2).

Molecular genetic identification

Randomly amplified polymorphic DNA (RAPD) markers

Table (4) and Fig.(6) show the results of total amplified fragments (TAF), amplified fragments (AF) and specific markers (SM) for the three genotypes of *Swietenia mahagoni* using RAPD-PCR analysis with five random primers. A total number of 42 DNA fragments were detected, in which 26 (61.90%) were polymorphic fragments. However, 16 bands were common (monomorphic) for the three genotypes. Polymorphism levels differed from one primer to another. The results found that (OP-C12 and OP-A10) primers exhibited high levels of polymorphism (80.00 % and 77.78 %) respectively. While, (OP-B07) primer exhibited moderate level of polymorphism (63.64%) and the other two primers (OP-A07) and (OP-B11) represented the lowest level (40.00% and 28.57 %) as exhibited in Table (4). The lowest number of polymorphic fragments was detected for primer OP-A07 and OP-B11 (2 out of 5 amplified bands and 2 out of 7 amplified bands) respectively, while the highest number of polymorphic fragments was detected for primer OP-C12 (8 out of 10 amplified bands). The lowest number of polymorphic fragments was detected for primer OP-D09 and OP-D01 (8 out of 10 amplified bands and 9 out of 17 amplified bands) respectively, while the highest number of polymorphic fragments was detected for primer OP-Z03 (22 out of 24 amplified bands). Cultivar-specific markers generated from RAPD-PCR analysis are shown in Table (4). Eighteen out of 42 RAPD-PCR fragments were found to be useful as cultivar-specific markers. The largest number of RAPD-PCR markers was scored for Giza tree genotype (9 markers), while the lowest (4 markers) was scored for Alexandria tree genotype. At the meantime, the highest number of RAPD-PCR cultivar-specific markers was generated by primer OP-A10 (6 markers), while the lowest number (1 marker) was generated by primer OP-B11.

Table (4): Species-specific RAPD and ISSR markers for three genotypes of Swietenia mahagoni.

Primer Name	Range of MS*	TAF*	MF*	PF*	SM* (bp)	Polymorphism (%)
RAPD primers						
OP-A07	489-895	5	3	2	1 (0) - (0) - (811)	40.00
OP-A10	400-1733	9	2	7	6 (0) - (457) - (1733,975,1440,586,466)	77.78
OP-B07	315-1314	11	4	7	5 (711) - (315-648) - (532-769)	63.64
OP-B11	297-1385	7	5	2	1 (579) - (0) - (0)	28.57
OP-C12	249-590	10	2	8	5(311,399)-(249,489)-(370)	80.00
Total RAPD		**42**	**16**	**26**	**18**	
ISSR primers						
HB-08	223-852	10	4	6	1 (0) - (0) - (598)	60.00
HB-09	281-1160	15	3	12	9 (874,466) - (926,543,502,418) - (1160,497,432)	80.00
HB-10	225-750	7	3	4	3 (750) - (730,225) – (0)	57.14
HB-11	303-1036	10	4	6	6(1036,933,490) - (443) - (528,460)	60.00
HB-12	253-921	12	3	9	7 (921,842,453) - (824) - (328,293,253)	75.00
HB-15	285-1038	8	6	2	2(703) – (285) – (0)	25.00
Total ISSR		**62**	**23**	**39**	**28**	
Total		**104**	**39**	**65**	**46**	

Inter simple sequence repeats (ISSR) markers

The six ISSR primers succeeded in amplifying DNA fragments for the three genotypes of *Swietenia mahagoni* (Fig. 7). Polymorphism levels differed from one primer to another, *i.e.* HB-09 primer exhibited high level of polymorphism (80.00%), while, HB-15 primers exhibited lowest level of polymorphism (25.00%) as exhibited in Table (4). The number of total amplified fragments (TAF), polymorphic fragments (PF), monomorphic fragments (MF) and specific markers (SM) for each primer of the six primers are shown in Table 4. HB-08 Primer showed ten DNA fragments with molecular size ranging from 223 to 852bp (Fig. 4 and Table 4), six fragments were polymorphic (60.00 %), only one band was positive species-specific markers at (598bp) for Giza tree genotype. However, HB-09 primer showed fifteen DNA fragments with molecular sizes ranging from 281 to1160 bp, twelve fragments were polymorphic (80.00 %) and nine of them were positive species- specific markers at (874 and 466 bp) for Alexandria tree genotype, (926,543,502 and 418 bp) for Nobaria tree genotype and (1160,497 and 432bp) for Giza tree genotype. HB-10 primer showed seven DNA fragments with molecular size ranging from 225 to750 bp, four fragments were polymorphic (57.14 %), and three of them were positive species- specific markers at (750 bp) for Alexandria tree genotype and (730,225 bp) for Nobaria tree genotype. HB-11 primer showed ten DNA fragments with molecular size ranging from (303-1036 bp), six fragments of them were polymorphic (60.00

%), and six of them were positive species-specific markers at (1036,933,490 bp) for Alexandria tree genotype, (443 bp) for Nobaria tree genotype and (528 and 460 bp) for Giza tree genotype.

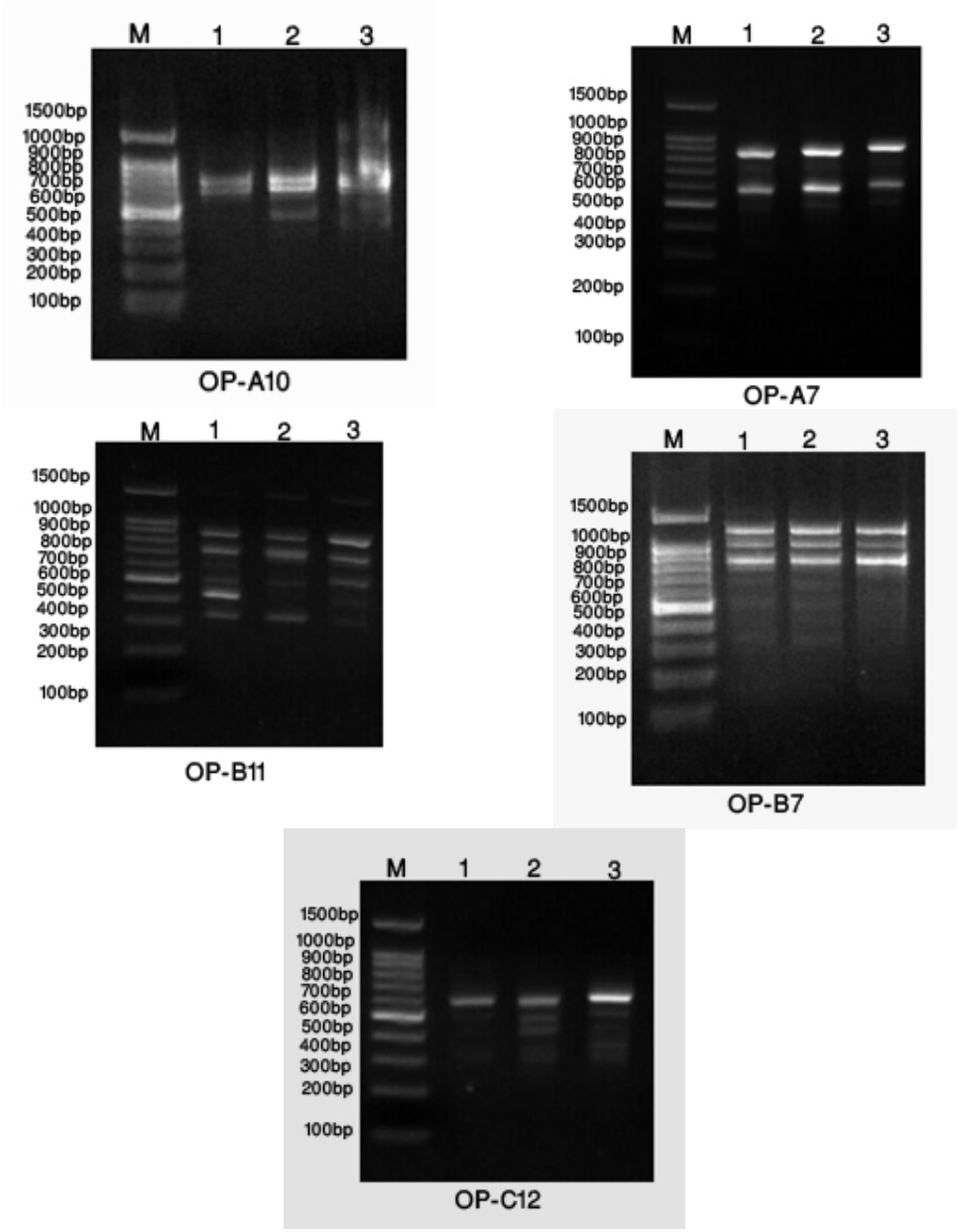


Fig. (6): RAPD-PCR analysis of three genotypes of Swietenia mahagoni. 1= Alexandria tree genotype, 2= Nobaria tree genotype, 3= Giza tree genotype.

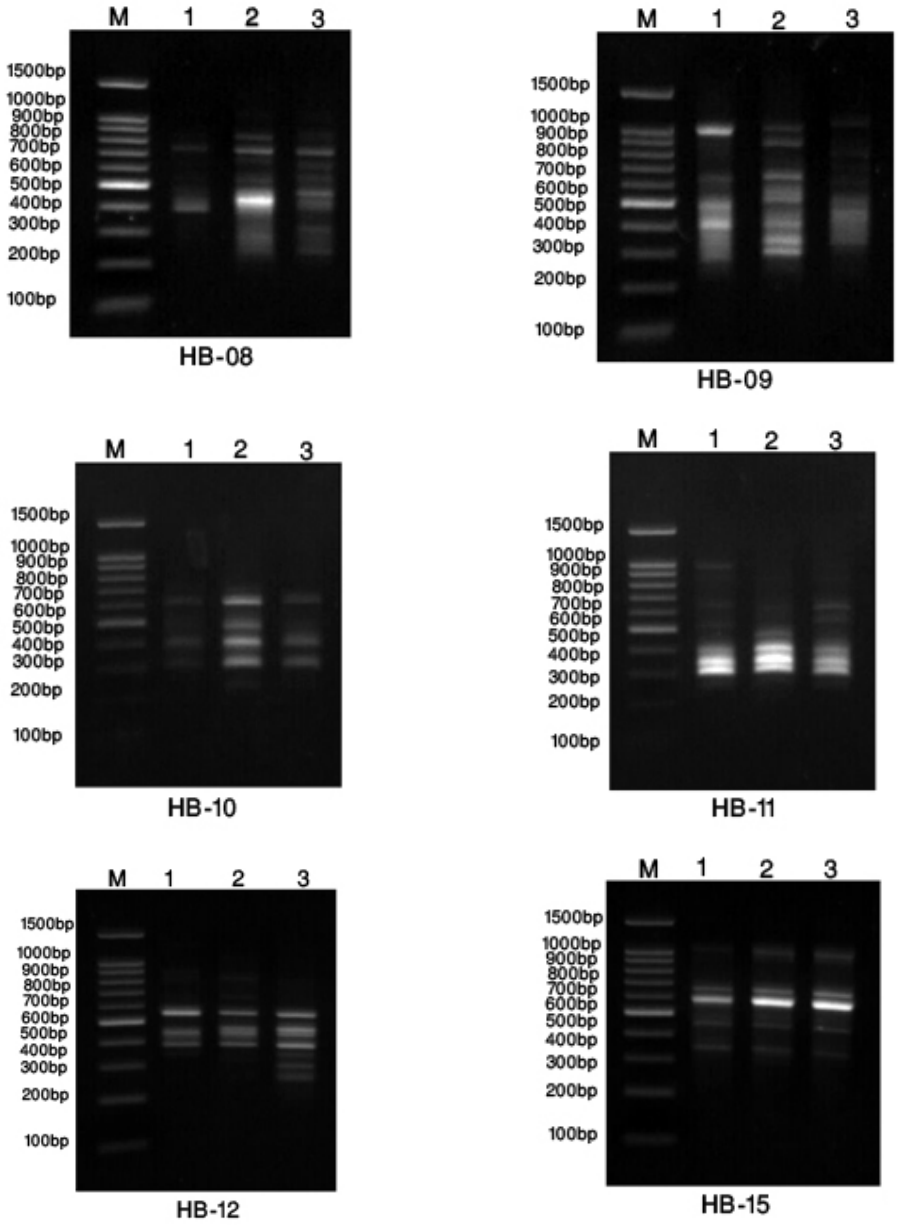


Fig. (7): RAPD-PCR analysis of three genotypes of Swietenia mahagoni. 1= Alexandria tree genotype 2= Nobaria tree genotype 3= Giza tree genotype.

Meanwhile, HB-12 Primer showed twelve DNA fragments with molecular size ranged from 253 to 921 bp, nine fragments of them were polymorphic (75.00 %), and seven of them were positive species- specific markers at (921, 842 and 453 bp) for Alexandria tree genotype, (824bp) for Nobaria tree genotype and (328, 293 and 253

bp) for Giza tree genotype. As for HB-15 primer, it showed eight DNA fragments with molecular size ranging from (285 to1038bp), only two fragments of them were polymorphic (25.00 %), and two of them were positive species- specific markers at (703 bp) for Alexandria tree genotype and (285 bp) for Nobaria tree genotype. In this work, we compared the applicability of ISSRs and RAPDs as genetic markers to characterize the three genotypes of *Swietenia mahagoni*. The results found that, RAPD markers were approximately equal to the ISSR assay with regard to polymorphism detection, as they detected 61.90 % as compared to 62.90 % for ISSR markers. This is in contrast to the results obtained for several other plant species like in wheat (Nagaoka and Ogihara, 1997) and *Vigna* (Ajibade *et al.*, 2000). The number of total polymorphic fragments is higher for ISSR than RAPDs. In fact, the ISSRs have a high capacity to reveal polymerphism and offer great potential to determine intra and inter genomic diversity as compared to other arbitrary primers like RAPDs (Zietkiewicz *et al.*, 1994).

Anatomical study

Fig. (8)
Nobaria location

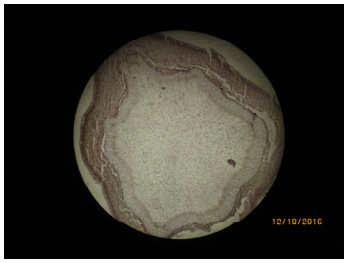


Fig.(9)
Alexandria location

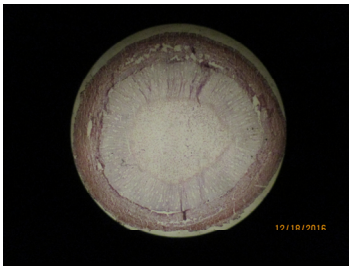


Fig. (10)
Giza location

Fig. (8:10): The cross section in the mahagoni stem cuttings from three different locations; Alexandria, Nobaria, and Giza with a magnification power of 32x. As shown, the xylem area in Nobaria cutting is larger than the other two locations and this may be attributed to the environmental conditions.

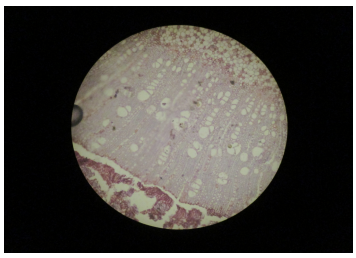


Fig. (11)
Nobaria location

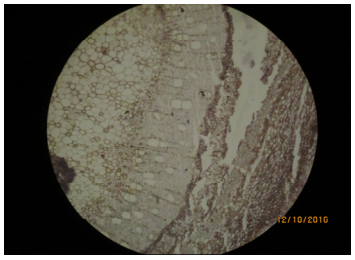
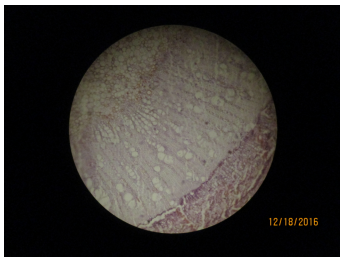


Fig.(12)
Alexandria location



Fi.g (13)
Giza location

Fig. (11 : 13): The xylem zone with a higher magnification power (125x). It is obvious that there are differences among the three locations, mainly the number of vessels. The number of vessels are greater in Nobaria xylem zone than those in Alexandria and Giza cuttings.

Yilmaz *et al*. (2008) examined the relationships between some environmental factors, altitude and physical soil properties and anatomical features of *Quercus pontica* C. Koch wood. They found that the physical soil properties (sand, silt and clay) affecting the "available water capacity" value of soil, which alter the microscopic properties of wood. They also indicated that there were significant relationships between soil organic and wood anatomy.

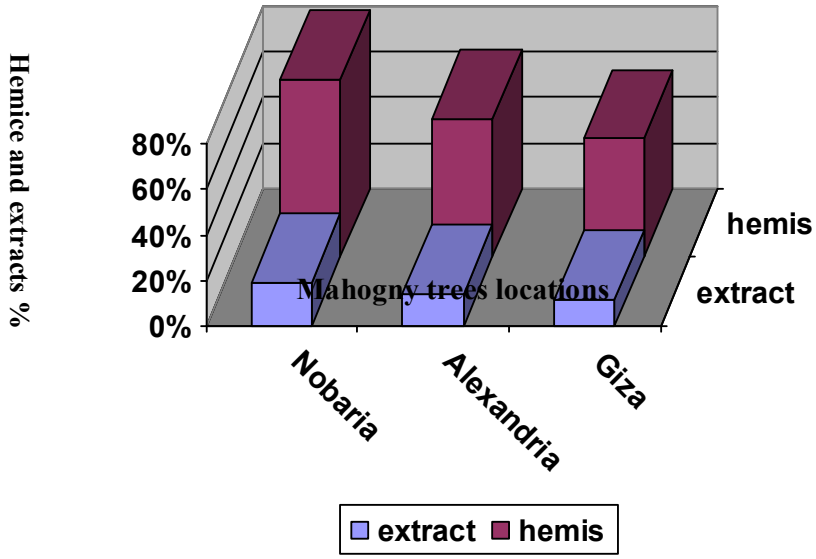


Fig. (14): Effect of the mahagoni (Swietenia mahagoni) tree location on wood chemical characteristics.

Wood chemistry

Wood extract

Data presented in Fig (14) indicated that, the high level of variation was on Nobaria mahagoni tree station, which recorded high extract percentage (19.40%) against Giza which recorded (11.8%) that had the lowest percentage.

Hemicellulose

Data in Fig (14) showd that the highest amount of hemicellulose was in Nobaria (78.18%) compared with the other two locations, which they recorded (60.92%-52.04 %) for Alex. and Giza, respectively.

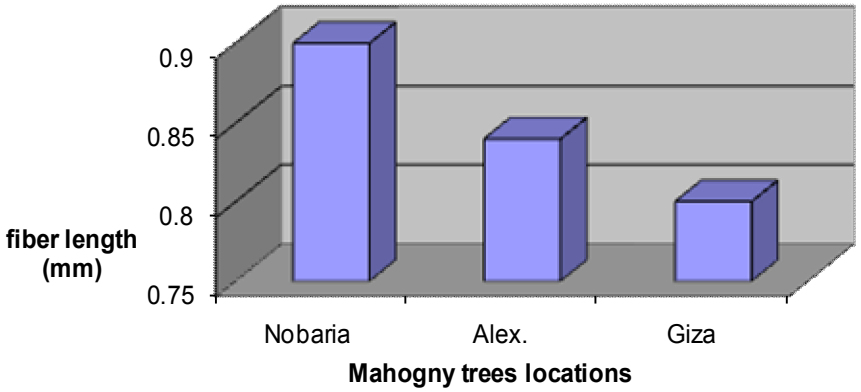


Fig. (15): Effect of the mahagoni (Swietenia mahagoni) tree location.

Data presented in Fig. (15) revealed that, Nobaria recorded the highest significant fiber length against the two other locations, where the difference between them was not significant. In this concern, Yilmaz *et al*. (2008) found that there were significant relationships between soil organic and wood anatomy and the physical soil properties (sand, silt and clay) affecting the microscopic properties of wood.

Leaflets area

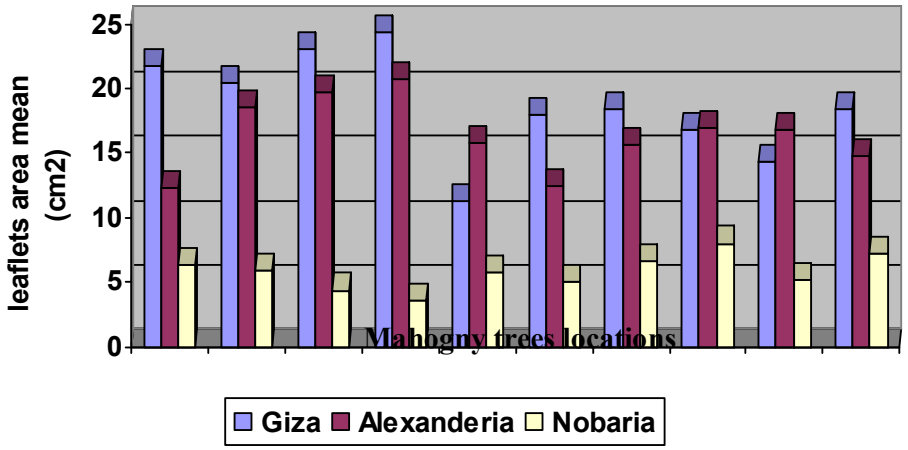


Fig. (16): Effect of three different regions on Swetenia mahagoni leaflets area mean.

Data presented in Fig. (16) showed that mahagoni leaflets showed that lower mean leaflets area at Nobaria station (3.5532 cm^2) compared to Giza and Alex. (24.32 - 20.672 cm^2), respectively. Spatial patterns of variation in the structure and productivity of

coastal plant communities have primarily been explained by the effects of steep environmental plant morphology and physiology (Oosting and Billings, 1942; Boyce, 1954; Levy, 1990; Ehrenfeld, 1990 and Young *et al*., 1994).

CONCLUSION

A pattern resistant genotype of *Swietenia mahagoni*, showed good tolerance to unfavourable environmental conditions (salinity and calcareous soil) was observed grown at Nobaria, Horticulture Research Station, Egypt، planted almost 30 years ago. In this paper، wood chemistry, anatomy and morphological studies were done. In addition, biochemical, isozyme and molecular studies using RAPD and ISSR methods were evaluated in order to understand adaptability and tolerance mechanisms of this resistant genotype. Our results observed that, tree which grown in salinity (Nobaria tree genotype) conditions had the highest amount of total phenolics, total flavonoids and total antioxidant compared to trees grown in non-saline conditions. Besides, it showed the highest amount of both wood extract and hemicellulose. Moreover, it had the tallest fiber length compared to the other two locations. Finally, the xylem area in Nobaria cutting was larger than the other two locations.

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We wish to express our sincere thanks, deepest appreciation and greatest respect to Prof. Dr. Ahmed Mahmoud Abd El Dayem Prof. of Timber Trees and Forestry Research Dep., Horticulture Research Institute, Agric. Research Center, for his help, valuable guidance and kind support. Moreover, he suggested the idea of this work and he cultivated the mahagoni tree.

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الملخص العربي

تقييم ٣ تراكيب وراثية لأشجار الماهوجنى الأسبانى

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بدأ استخدام خشب الماهوجاني منذ زمن طويل منذ القرن الثامن عشر في جزر الكاريبي. وقد استخدم لأول مرة كبديل لخشب البلوط لبناء السفن. ومع ذلك ، فإن هذه الشجرة مهددة بالانقراض الآن. وهذه الدراسة تلقى الضوء على شجرة الماهوجنى الفائقة الخصائص التى أبدت تحمل للظروف الغير مناسبة، والتى ظلت مستمرة على قيد الحياة من ضمن ١٠٠ شجرة أخرى زرعت معها فى منطقة النوبارية. وقد صُممت هذه الدراسة لتشخيص الصفات المورفولوجية ولشرح التشابه والتنوع من حيث الأنزيمات ، و RAPD, ISSR لثلاثة تراكيب وراثية من *Swietenia mahagoni* للمقارنة بينهما والتحقيق في مصدر جيني جديد. تم الحصول على ثلاثة تراكيب وراثية مختلفة من *Swietenia mahagoni* من محافظة الإسكندرية ، ومشتل قسم الأشجار الخشبية بمعهد بحوث البساتين بمحافظة الجيزة ، ومنطقة شمال التحرير بمحافظة البحيرة. وقد تم تحديد خصائص التربة. وتم استخراج المركبات المضادة للأكسدة والبولفينولات وتحديدها وتم عمل تحليل كهربائي للمتشابهات الانزيمية Isoenzyme للعينات. تم عمل توصيف لثلاثة تراكيب وراثية مختلفة من *Swietenia mahagoni* على المستوى الجزيئي. أيضا ، تم تحديد تكرار التتابع. أظهرت النتائج أن محتوى كربونات الكالسيوم للعينات وجد أنه مرتفع مما يسبب تثبيت الفسفور. تم الحصول على إجمالي المركبات الفينولية، ومحتوى الفلافونويد، ونشاط مضادات الأكسدة بنسبة كبيرة. كذلك لوحظ أعلى محتوى للفلافونيدات وهو ٤.٢٤ ملجم / ١٠٠ جم في أوراق الشجرة فى التركيب الوراثى من الإسكندرية. أظهر التحليل الكهربي لأنماط البيروكسيديز والبوليفينيل أكسيديز فى الثلاثة أنواع وجود بعض الاختلافات في كثافة النطاقات مقارنة مع بعضها البعض و كل نطاقات البيروكسيديز الثلاثة ، وتم ملاحظة وجود ثلاث مناطق بكثافات مختلفة في الثلاث عينات. بالنسبة لنمط تباين مادة البوليفينول أوكسيديز ، لوحظ وجود نطاقيْن لهما شدة مختلفة بين بروفيلات التراكيب الوراثية الثلاثة من *Swietenia mahagoni* . ولقد كشفت التحاليل الكيميائية للأخشاب أن مستوى التباين العالى وجد فى شجرة الماهوجني التى توجد فى محطة النوبارية والتي سجلت نسبة مستخرجات عالية مقارنة بالجيزة التي سجلت أقل نسبة. أظهر تحليل الهيميسليلوز أن الشجرة الموجودة بالنوبارية سجلت أعلى نسبة مئوية مقارنة بالأسكندرية والجيزة. أظهر تحليل طول الألياف أن الشجرة الموجودة فى النوباريه سجلت معنوياً أعلى طول للألياف مقارنة بالموقعين الآخرين. في الختام ، تم تحديد أوجه التشابه بين التراكيب الوراثية الثلاثة ، و أيضا تحديد الاختلافات بينهم وتم إثبات أن هذا النموذج من الجينوم الماهوجنى والمزروع فى النوبارية بمحطة بحوث البساتين فى مصر أظهر تحمل جيد للظروف الغير مناسبة (الملوحة والأرض الكلسية).