Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, Egypt
27(4), 2273-2290, 2019
Website: http://ajs.journals.ekb.eg

MORPHOLOGICAL, CHEMICAL CHARACTERS AND GENETIC ANALYSIS DISCRIMINATION OF FIVE NATURALIZED *POPULUS* SPECIES INHABITING FOUR GOVERNORATE TERRITORIES OF EGYPT

[181]

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Received 7 October, 2019 Accepted 12 November, 2019

ABSTRACT

Populus is a fast growing tree that attract the attention of people. This genus provides environment protection due to their ability in carbon sequestration, phytoremediation and wildlife inhabitant. *Populus* species are widely used as a source of wood, veneer, paper and bioenergy. This survey study aimed to identify the current status of *Populus* species inhabiting four different governorates of the Egyptian territories. In this survey, four different locations were surveyed, i.e. Cairo, Giza, Qaliobia and Gharbia governorates. According to the site survey, five species of *Populus* were found. Research findings indicate that the length and diameter of the trees varied according to various species in various localities. Selected trees of various species were subjected to different morphological, chemical parameters and genetic assessments. *P. deltoides* from Giza in addition to *P. deltoides* and *P. nigra* from Cairo gave somewhat close relation in their vegetative parameters. Furthermore, both *P. nigra* and *P. alba* from Gharbia location gave high similarity due to overall vegetative parameters and also both *P. euramericana* female from the Gharbia and *P. nigra* from Qaliobia. Genetic diversity was analyzed using molecular markers. Three different Inter-simple sequence repeat (ISSR) primers were used for the reaction (17899A, 17899B and HB13). ISSR primers markers yielded 67.5% polymorphic loci among the surveyed species and cluster analysis enabled separation of these populations on the basis of their genetic distances. *P. euramericana* female and male from Giza showed close relation at morphological level. From this study it can be concluded that: firstly; leaf parameters i.e. leaf area width and length were suitable for identifying the differences among species, and secondly; genetic analysis using molecular markers was enough to discriminate between species. Our results finally suggest that *Populus* species possess genetic variation and can adapt to new environmental conditions, which in its self-valuable information would potentially lead to promising applications.

Keywords: Egypt, Survey study, Woody trees, Poplar, *Populus*, Morphology, Genetic analysis, ISSR

INTRODUCTION

Poplar (*Populus* spp.) is regarded as a group of species growing all over the world. Due to its fast-growing feature and acceptable quality of timber; it plays significant roles in silviculture. Therefore, paying attention to its characteristics along with gathering more information on it can enable us to apply and treat correctly. To our knowledge no survey was conducted up till now on the importance of popular trees diversity and various morphological and biometric analyses in Egypt.

Plant surveys are designed to search in geographic areas to determine the presence of exotic species, like *Populus* amongst other species and evaluate its their habitat suitability (**Haber and Network, 1997**).

Poplar include 33 species belonging to the family *Salicaceae*. The poplar species are native to North America. The wood of poplars is relatively soft and hence is mostly used to make cardboard boxes, crates, paper, and veneer (**Isebrands & Richardson, 2000**)

Some Populations of *Populus* face severe threats. Three main factors have been recognized. The first one is the alteration of riparian ecosystems throughout the species' distribution area. Second, faster growing hybrid poplars have been planted to replace black poplar or they have just been removed due to over-exploitation. Finally, there is the potential threat of introgression from cultivated poplars such as the male clone 'Italica' (**Cagelli and Lefèvre 1995, Lefèvre et al 1998**).

Leaf morphology may offer precise differentiation because it varies remarkably among species and within species with respect to structure, dimensions, types of margins, form, size of petiole, venation pattern, dry weight per unit area, moisture content, canopy, stomata density, presence of trichomes and cuticular composition (**Rodriguez et al 2016**).

Interesting enough, genetic discrimination analyses using various techniques, i.e. AFLP or ISSR represent rather new insight for curious differentiation among species (**Bandyopadhyay et al 2013**). Inter simple sequence repeat (ISSR) requires very small amount of template and is convenient in result recording and highly reproducible (**Zietkiewicz et al 1994**).

Accordingly, the aim of this study was to conduct basic research to identify and find out similarities and differences amongst *Populus* species surveyed at the morphological, chemical and genetic levels.

MATERIAL AND METHODS

Location and duration

A rapid reconnaissance survey was carried out at four research stations belonging to the Agriculture Research Center at four different governorates of Egypt, viz. Cairo, Giza, Qaliobia and Gharbia. Survey result data in **Table (1)** demonstrate the presence of five species of *Populus* at the Horticulture Research Stations, located in four governorates from which plant material were collected in June and August of 2016.

Table 1. Naturalized *Populus* species at research stations in four governorates of Egypt

Governorate	Research Station	*Populus* species
Giza	Horticulture research institute	*Populus euramericana* ♀ *P. euramericana* ♂ *P. deltoides*
Cairo	Zohria garden	*P. nigra*
Qaliobia	Horticulture research station in Qanater	*P. nigra*
Gharbia	Agriculture research station in Gemiza	*P. nigra* *P. deltoides* *P. alba* *P. euramericana* ♀

Data collected

Morphological characteristics

- **Tree height** was estimated with the use of a clinometer**.**
- **Tree** diameter **at Breast Height** (DBH)**,** i.e. the stem diameter measured in cm at 1.5 m above ground level. Number of branches along the main trunk were counted.
- **Number of branches.**
- **Leaf area, width and length of leaf,** they were estimated selecting completely grown leaves from the middle of branches. Leaves were photographed using a digital camera as presented in **Plate (1)** then resulted photos were elaborated using Image J program (Schneider *et al*., 2012) which calculated individual leaf area, width and length.

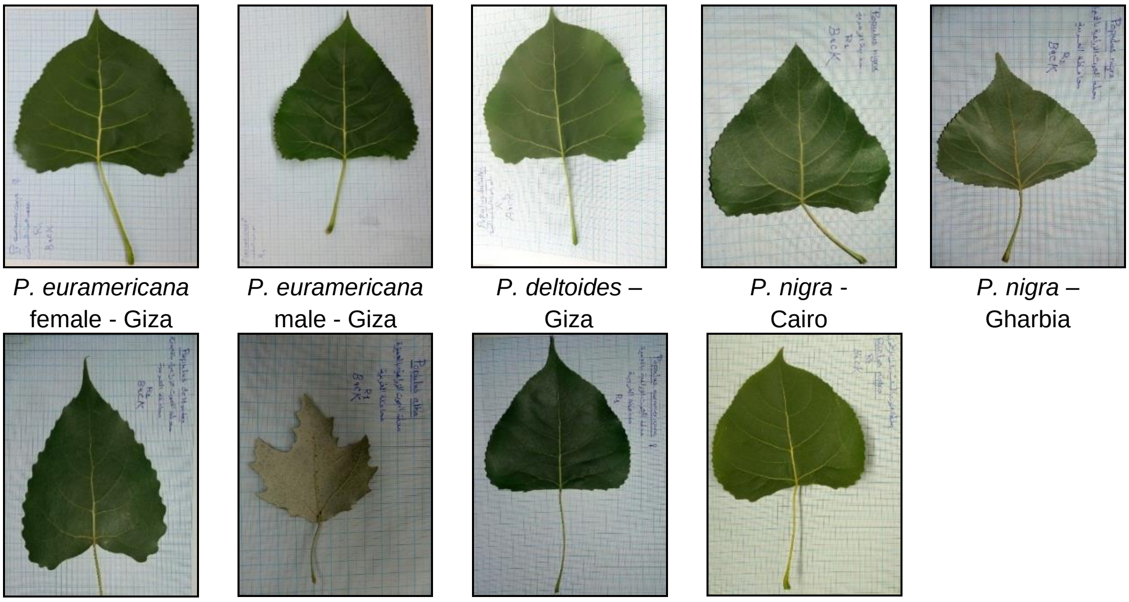

Plate 1. Photographed mature leaves of surveyed *Populus* species from four governorates in Egypt, viz. Giza, Cairo, Qaliobia and Gharbia in June-August 2016

- **Leaf fresh and dry weight,** 20 leaves were collected then weight to obtain fresh weight then oven-dried at 70 °C to estimate dry biomass.
- **Leaf greenness** was estimated using SPAD**.**

Internal chemical analysis

The content of N, P, K, Ca and Mg elements were determined in oven dried samples of leaves by digestion using concentrated H_2SO_4. N percentage was determined in the digested solution by the modified microkjeldahl method as described by **Plummer (1971)**. P percentage was determined calorimetrically according to the method of **Jackson (1958)**. K percentage was determined against a standard using a flame photometer **(Piper, 1950)**. Ca and Mg percentage was determined by an atomic absorption spectrophotometer, according to **David (1959)**.

DNA Extraction

DNA extraction was applied for the nine different treatments of *Populus* plant. DNA extraction followed CTAB method according to **Doyle and Doyle (1990)** and modified by **Edwards et al (1991)**.

Molecular Marker Bioassay Analysis (ISSR)

The ISSR molecular marker was applied to calculate the variation in Populus plant **(Meyer et al 1993)**. Three different ISSR primers were used for the reaction (17899A, 17899B and HB13) as described in **Table 2**.

Statistical Analysis

Survey data were statistically analyzed according to the separation among means using Duncan multiple range test (**Duncan, 1995**). To identify the relation between each individual characteristic of various *Populus* species, the correlation was analyzed using the CoStat.

Image results from electrophoresis were analyzed by the presence of a band which scored as 1, whereas the absence of the band was coded as 0. A pair wise similarity matrix was generated using Jaccard's similarity coefficient and using the unweighted pair group method with the arithmetic averaging algorithm (UPGMA), cluster analysis was performed to develop a dendrogram. These calculations were carried out using Bio-Rad Quantity one (4.6.2) as prescribed by **Li et al (2008)**.

Table 2. List of primer names (ID) utilized, their nucleotide sequences in the ISSR analysis

No	Primer name	Primers nucleotide sequence	GC%	Temperature (°C)
1	17899A	5’-CACACACACACAAG-3’	50	42
2	17899B	5’-CACACACACACAGG-3’	57.1	50
3	HB13	5’-GAGGAGGAGGC-3’	72.7	38

RESULTS

Morphological parameters

Morphological parameters were demonstrated in **Fig. 1**. Diameter of trees at breast height (DBH) showed significant variation among different species. *P. deltoides* and *P. euramericana* female Giza and *P. deltoide*s from Gharbia gave the largest DBH (54.1, 49.1 and 48.4 cm, respectively). Whereas, the lowest DBH were obtained from *P. euramericana* female in Gharbia and *P. nigra* in Qaliobia (22.3 and 25.5 cm, respectively). Number of tree branches on different species were varied, the highest value was found in both *P. euramericana* female from Giza and *P. nigra* from Cairo (9.8 and 9 branches/ tree, respctively). Whereas, *P. euramericana* female from Gharbia showed the lowest number of branches (3 branches). As far as tree height was concerned, *P. nigra* from Cairo and *P. euramericana* female from Giza showed the tallest height among different species surveyed (17 and 16.33 m, respectively). Whereas, *P. euramericana* female from Gharbia showed the lowest value of tree height (6 m). Average of leaf area (LA) showed variation among species. Both female and male of *P. euramericana* from Giza were superior in their leaf area/leaf (225 and 210 cm^2/leaf, respectively). Whereas, the smallest leaf area was found in *P. alba* from Gharbia (35 cm^2/leaf). Linearly with LA leaf width and length showed similar trend. Both female and male of *P. euramericana* from Giza were superior in their leaf width and length (25.38 × 17.20 cm and 28.7 × 17.4 cm). Whereas, the smallest value was found in *P. alba* from Gharbia (11.8 × 7.6 cm). The fresh and dry weight of collected leaf samples showed relative variation among the surveyed species in different governorates. The highest fresh and dry weight was found in *P. euramericana* male from Giza (3.6 and 1.2 g/leaf). Whereas, the lowest fresh weight was found in *P. alba* from Gharbia (1 g/leaf), in respect order and lowest dry weight was found in *P. nigra* from Cairo (0.4 g/leaf).

Greenness of leaves

SPAD readings in **Fig. 2** recorded highest value in the *P. alba* from Gharbia governorate (53.35). *P nigra* plants in surveyed governorates gave close insignificant values (44.65, 45.90 and 47.70) in Qaliobia, Cairo and Gharbia, respectively). Moreover, results obtained in case *P. deltoides* and *P. euramericana* male and female were more or less equal with lowest values regardless of governorate location (refer to **Fig. 2**).

Leaves Chemical Analysis

N, P and K % concentrations, in leaf samples of *Popolus* species surveyed are shown in **Figure 2**. The amount of N averaged 2% in sampled leaves and the highest % of N was found in *P. nigra* from Qaliobia governorate (2.27%). Whereas, the lowest N% was found in *P. nigra* from Gharbia (1.67%). The amount of P ranged from 0.15 to 0.55% in leaf samples. P showed higher percentage for all species in Gharbia governorate (0.51, 0.48, 0.48 and 0.58% in *P. deltoides*, *P. euramericana* female, *P. alba* and *P. nigra*, respectively) when compared to the other species in other three governorates surveyed. The amount of K was between 0.6-1.1% in sampled leaves. Without any significant difference among surveyed species in all governorate locations. Mg and Ca elements showed similar trends in which the lowest values (0.169 and 0.114, respectively) were obtained from species *P. euramericana* female and *P. alba* grown in Gharbia governorate and showed higher percentage in Giza governorate *P. euramericana* male (0.528 and 1,07, respectively).

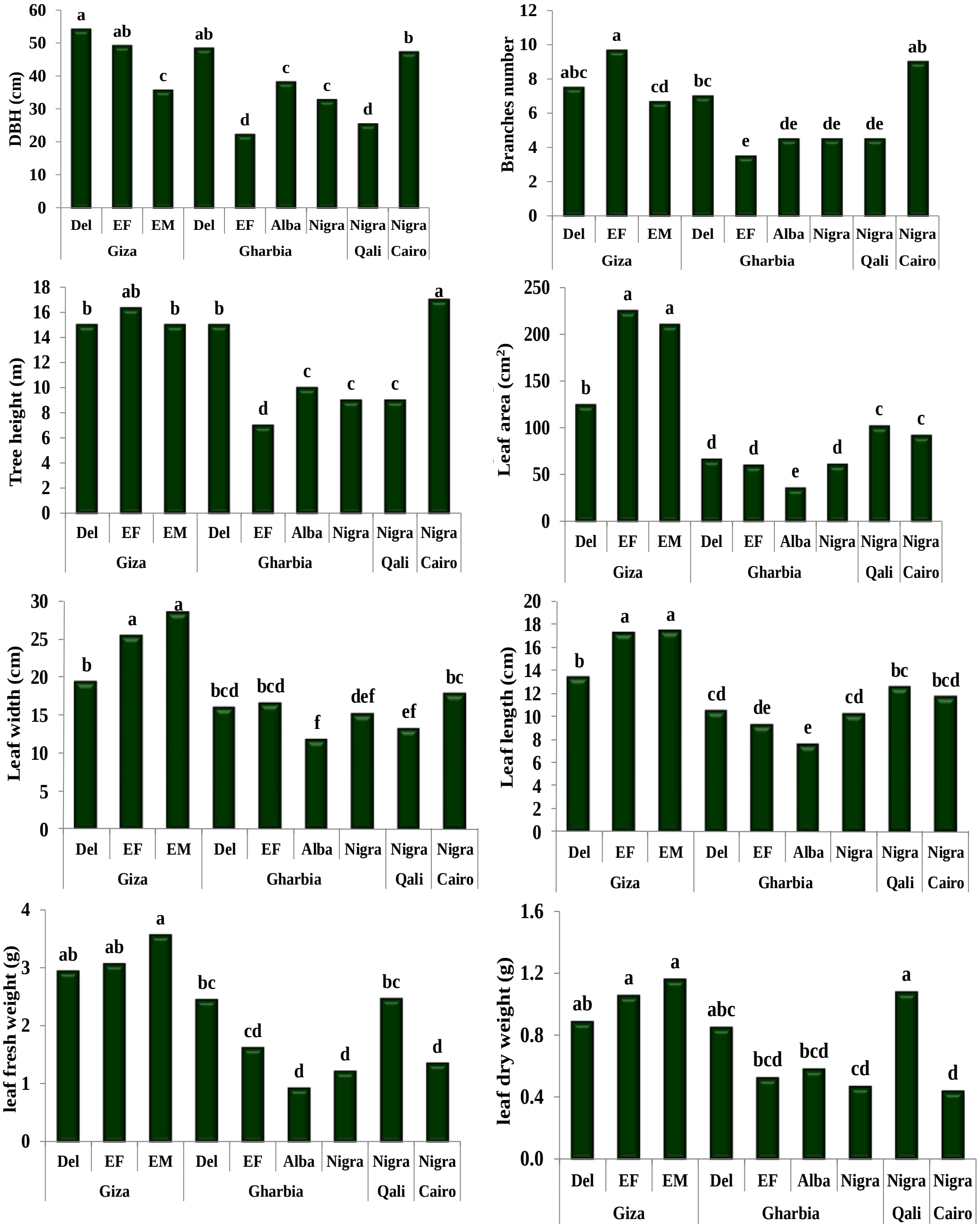

Fig. 1. Vegetative parameters of different surveyed *Populus* species in four governorates of Egypt. Abbreviations: Del: *P. deltoides*, EF: *P. euramericana* female, EM: *P. euramericana* male, Alba: *P. alba*, Nigra: *P. nigra*, Qali: Qaliobia.

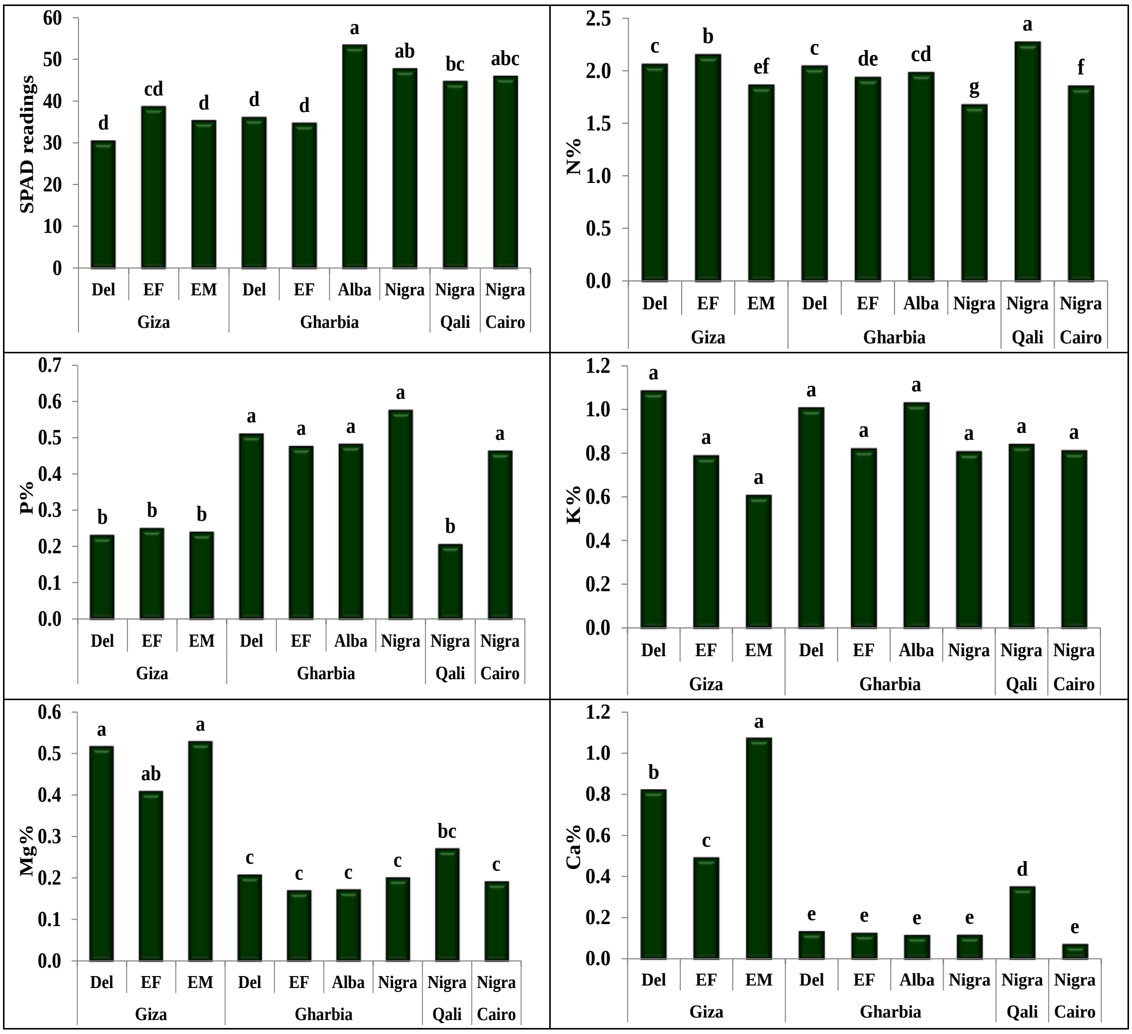

Fig. 2. Internal chemical content of N, P, K, Mg and Ca and greenness colour of leaves SPAD value in sampled leaves of different surveyed *Populus* species in four governorates of Egypt.
Abbreviations: Del: *P. deltoides*, EF: *P. euramericana* female, EM: *P. euramericana* male, Alba: *P. alba*, Nigra: *P. nigra*, Qali: Qaliobia.

According to the coefficients of correlation, the relation among the examined morphometric and chemical characters varied (**Table 3**). DBH and number of branches were significantly correlated with the increase in tree height. A high correlation was found also among length of the leaf blade, width of the leaf blade and leaf area suggesting that these characters share similar info and each could be an indicator for the same information about the difference amongst populations. The increase in Ca content was positively correlated with various leaf parameters. The P% showed negative correlation with leaf parameters and N%. Leaves dry weight also negatively correlated with Mg content and SPAD value.

Neighbor-joining clustering trees constructed from available morphological and chemical analysis data showed the relation among different studied species (**Plate 2**). *P. euramericana* female and male from Giza showed close relation at morphological level. In addition, *P. deltoides* from Giza, *P. deltoides and P. nigra* from Cairo gave high similarity. Furthermore, both *P. nigra* and *P. alba* from Gharbia gave high similarity as was also both *P. euramericana* female from Gharbia and *P. nigra* from Qaliobia.

Table 3. Correlation coefficients* among all phenology traits across some *Populus* species inhibiting in four governorates of Egypt

	Bn	LDw	SPAD	Tree height	N%	P%	K%	Mg%	Ca%	Leaf area	Lbw	Lbl
DBH	0.84	0.29	-0.25	0.86	0.10	-0.13	0.42	-0.10	0.22	0.30	0.31	0.30
	0.0049	0.4513	0.5216	0.0028	0.7972	0.7331	0.2578	0.7986	0.568	0.4333	0.4242	0.432
Bn		0.46	-0.28	0.95	0.15	-0.33	-0.06	-0.28	0.30	0.63	0.60	0.63
		0.2183	0.472	0.0001	0.6912	0.3859	0.8783	0.4623	0.4401	0.0702	0.0909	0.0699
LDw			-0.73	0.51	0.43	-0.80	-0.28	-0.81	0.85	0.84	0.78	0.87
			0.0249	0.1631	0.2444	0.0089	0.4699	0.0083	0.0033	0.0045	0.0141	0.0021
SPAD				-0.32	-0.21	0.43	0.02	0.42	-0.59	-0.46	-0.57	-0.49
				0.4028	0.5939	0.2465	0.9543	0.2587	0.0953	0.217	0.1113	0.1822
Tree height					0.08	-0.31	-0.03	-0.25	0.40	0.59	0.61	0.61
					0.8347	0.4139	0.932	0.5144	0.2896	0.0953	0.0806	0.0839
N%						-0.65	0.30	-0.58	0.15	0.24	-0.06	0.23
						0.058	0.4277	0.1041	0.6913	0.5269	0.8835	0.5466
P%							0.19	0.98	-0.79	-0.75	-0.53	-0.77
							0.6267	0.0001	0.0117	0.0201	0.1413	0.0161
K%								0.23	-0.29	-0.54	-0.60	-0.55
								0.5547	0.4515	0.1294	0.0874	0.1227
Mg%									-0.82	-0.78	-0.57	-0.79
									0.0064	0.0125	0.1087	0.011
Ca%										0.77	0.76	0.79
										0.0146	0.0164	0.0107
Leaf area											0.92	0.98
											0.0004	0.0001
Lbw												0.90
												0.0008

DBH: Diameter at Breast Height, Lbw: width of the leaf blade, Lbl: length of the leaf blade, Bn: branches number, LDw: Leaf dry weight, Lbw: Leaf blade wide, Lbl: Leaf blade length.

* Correlation coefficient according to Pearson's test as described by **Benesty et al (2009)**

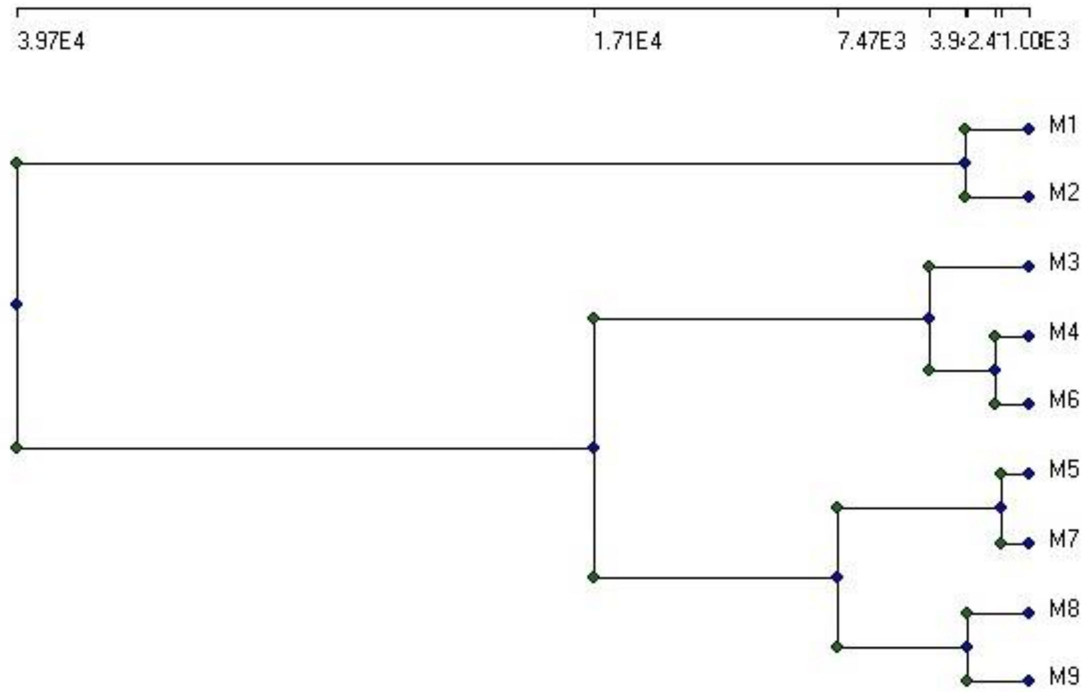

Plate 2. Dendogram from morphological data from field data collected from *Populus* species surveyed at four governorates of Egypt.

Abbreviations: M1: *P. euramericana* female Giza, M2: *P. euramericana* male Giza, M3: *P. deltoides* Giza, M4: *P. nigra* Cairo, M5: *P. nigra* Gharbia, M6: *P. deltoides* Gharbia, M7: *P. alba* Gharbia, M8: *P. euramericana* female Gharbia, and M9: *P. nigra* Qaliobia.

Genetic discrimination analysis

Initially, six ISSR primers were screened for polymorphism and based on the clear scorable band pattern, three primers (17899A, 17899B and HB13) were selected for DNA analysis of the species and were of good quality **(Plates 3, 5 and 7)**. These primers produced polymorphic, as well as, monomorphic bands when applied to the Poplar species understudied. A total number of 34 bands resulted from these primers. 13 bands with 17899A, 12 bands with 17899B and 9 bands with HB13 primer. The total number of polymorphic bands resulted from the three ISSR primers were 21 bands. These bands resulted in the total polymorphism percentage of 67.52%. The data for each primer were shown with dendograms in Plates (4, 6 and 8) for 17899A, 17899B and HB13 respectively, and illustrated in **Tables (4, 5 and 6)** respectively.

Climatic differences both affected the genetic content of the *Populus* species beside their original genetic structure and this was reflected on the plant behavior (both morphologically and physiologically) **(Table 7)**. For instance, there were 8 Unique bands apparent. Three unique bands for 17899A primer: 832 and 472 bp for *P. nigra* from Cairo and 277 bp for *P. alba*. Two unique bands with 17899B primer: 570, 441 and 212 bp with *P. alba* from Gharbia. Three unique bands with HB13 primer: 985 and 936 bp with *P. alba* from Gharbia and 486 with *P. nigra* Gharbia. So, *P. alba* from Gharbia was the most affected one with this variation.

The similarity coefficient values ranged from 0.342 to 0.723 using dice coefficient **(Table 8)**; showed a close relationship between M1 (*P. euramericana* Female Giza) and M8 (*P. euramericana* Female Gharbia) (0.723) and least genetic similarity between M6 (*P. deltoides* Gharbia) and M7 (*P. alba* Gharbia) (0.342). The genetic relationships among poplar species distributed in Egypt were analyzed by UPGMA method **(Plate 9)**. The dendrogram separated the poplar into two major groups genetic distance 0.48. In addition, the combination between genetic and morphological characteristics resulted in a clear relation among surveyed species **(Plate 10)**.

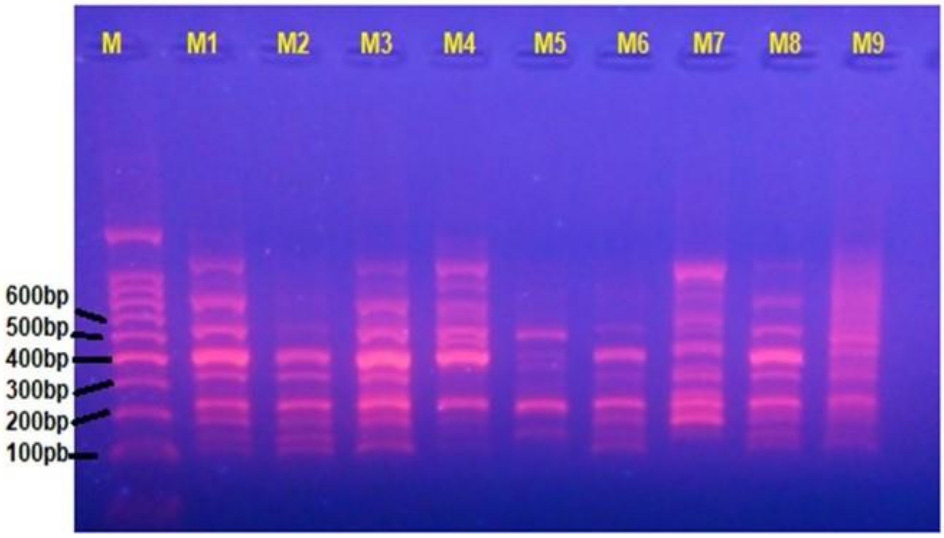

Plate 3. Gel electrophoresis of band patterns obtained from 17899A primer applied to nine samples collected samples of *Populus* species surveyed at four governorates of Egypt.
Abbreviations: M1: *P. euramericana* female Giza, M2: *P. euramericana* male Giza, M3: *P. deltoides* Giza, M4: *P. nigra* Cairo, M5: *P. nigra* Gharbia, M6: *P. deltoides* Gharbia, M7: *P. alba* Gharbia, M8: *P. euramericana* female Gharbia, and M9: *P. nigra* Qaliobia.

Table 4. Data analysis of band patterns of 17899A primer applied to nine samples of *Populus* plant species understudied at four governorates of Egypt.

Band no.	Mwt. (bp)	M1	M2	M3	M4	M5	M6	M7	M8	M9	Band type
1	923.182	1	0	1	1	0	0	1	1	0	P
2	832.033	0	0	0	1	0	0	0	0	0	U
3	700.000	1	0	0	0	0	0	0	1	0	P
4	658.141	0	0	1	1	0	0	0	0	0	P
5	523.318	1	1	1	1	1	1	1	1	1	M
6	472.871	0	0	0	1	0	0	0	0	0	U
7	405.618	1	1	1	1	1	1	1	1	1	M
8	326.489	1	1	1	0	0	1	1	1	1	P
9	277.703	0	0	0	0	0	0	1	0	0	U
10	224.565	1	1	1	1	1	1	1	1	1	M
11	170.850	1	1	1	1	1	1	1	1	1	M
12	113.431	0	1	0	0	0	1	0	0	0	P
13	88.159	0	1	1	0	0	0	0	0	0	P
Total no. of band for each sample		**7**	**7**	**7**	**8**	**4**	**6**	**7**	**7**	**5**	
Polymorphism%		**69.23%**									

Abbreviations: M1: *P. euramericana* female Giza, M2: *P. euramericana* male Giza, M3: *P. deltoides* Giza, M4: *P. nigra* Cairo, M5: *P. nigra* Gharbia, M6: *P. deltoides* Gharbia, M7: *P. alba* Gharbia, M8: *P. euramericana* female Gharbia, and M9: *P. nigra* Qaliobia.

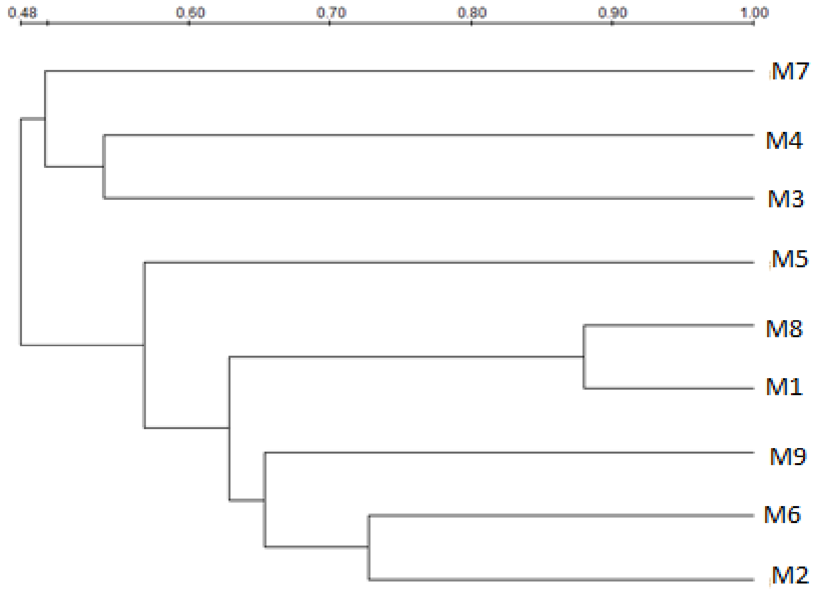

Plate 6. Dendogram for the differences among *Populus* species data surveyed at four governorates of Egypt according to the use of 17899A primer.
Abbreviations: M1: *P. euramericana* female Giza, M2: *P. euramericana* male Giza, M3: *P. deltoides* Giza, M4: *P. nigra* Cairo, M5: *P. nigra* Gharbia, M6: *P. deltoides* Gharbia, M7: *P. alba* Gharbia, M8: *P. euramericana* female Gharbia, and M9: *P. nigra* Qaliobia.

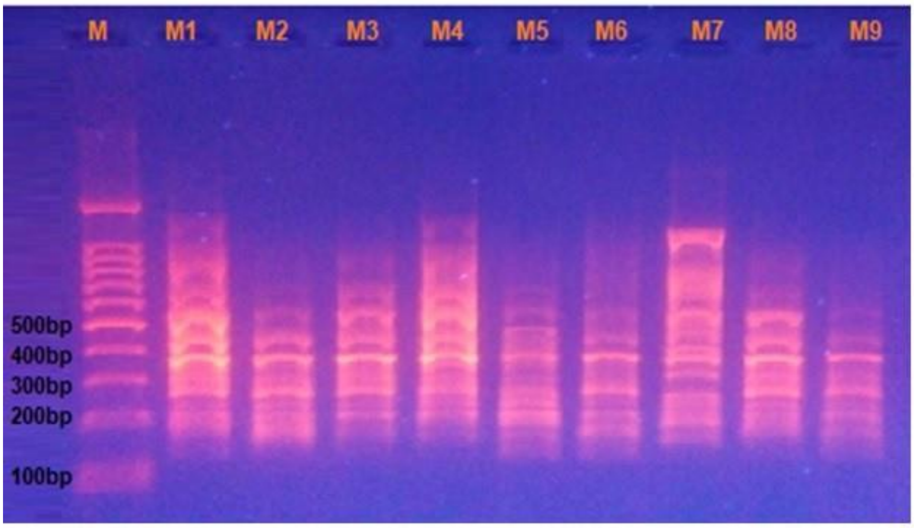

Plate 5. Gel electrophoresis of band pattern obtained from 17899B primer with collected samples of *Populus* species surveyed at four governorates of Egypt.
Abbreviations: M1: *P. euramericana* female Giza, M2: *P. euramericana* male Giza, M3: *P. deltoides* Giza, M4: *P. nigra* Cairo, M5: *P. nigra* Gharbia, M6: *P. deltoides* Gharbia, M7: *P. alba* Gharbia, M8: *P. euramericana* female Gharbia, and M9: *P. nigra* Qaliobia.

Table 5. Data analysis of band pattern of 17899B primer applied to nine samples of *Populus* plant species understudied at four governorates of Egypt.

Band no.	Mwt. (bp)	M1	M2	M3	M4	M5	M6	M7	M8	M9	Band type
1	700.000	1	0	1	1	0	0	1	0	0	P
2	570.895	0	0	0	0	0	0	1	0	0	U
3	516.852	1	0	1	1	1	0	0	0	0	P
4	441.020	0	0	0	0	0	0	1	0	0	U
5	411.314	1	1	1	1	1	1	1	1	1	M
6	330.193	1	1	1	1	1	1	1	1	1	M
7	265.640	1	1	1	1	1	1	1	1	1	M
8	212.542	0	0	0	0	0	0	1	0	0	U
9	169.349	1	1	1	1	1	1	1	1	1	M
10	102.811	0	1	0	0	1	0	1	1	1	P
11	87.055	1	1	1	1	1	1	0	1	0	P
12	80.107	0	0	0	1	0	1	1	1	1	P
Total no. of band for each sample		**7**	**6**	**7**	**8**	**7**	**6**	**10**	**7**	**6**	
Polymorphism%		**66.67%**									

Abbreviations: M1: *P. euramericana* female Giza, M2: *P. euramericana* male Giza, M3: *P. deltoides* Giza, M4: *P. nigra* Cairo, M5: *P. nigra* Gharbia, M6: *P. deltoides* Gharbia, M7: *P. alba* Gharbia, M8: *P. euramericana* female Gharbia, and M9: *P. nigra* Qaliobia.

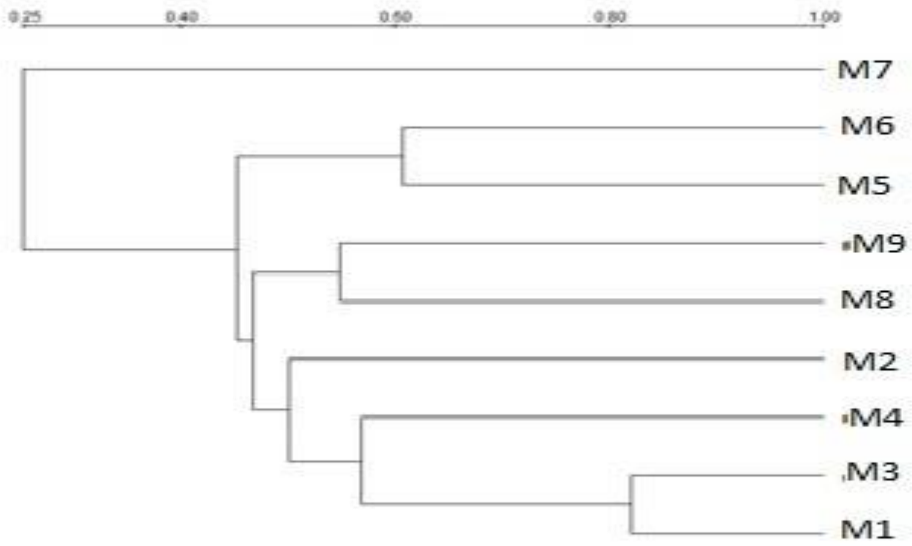

Plate 8. Dendogram for the differences among *Populus* species data surveyed at four governerates of Egypt according to the use of 17899B primer.
Abbreviations: M1: *P. euramericana* female Giza, M2: *P. euramericana* male Giza, M3: *P. deltoides* Giza, M4: *P. nigra* Cairo, M5: *P. nigra* Gharbia, M6: *P. deltoides* Gharbia, M7: *P. alba* Gharbia, M8: *P. euramericana* female Gharbia, and M9: *P. nigra* Qaliobia.

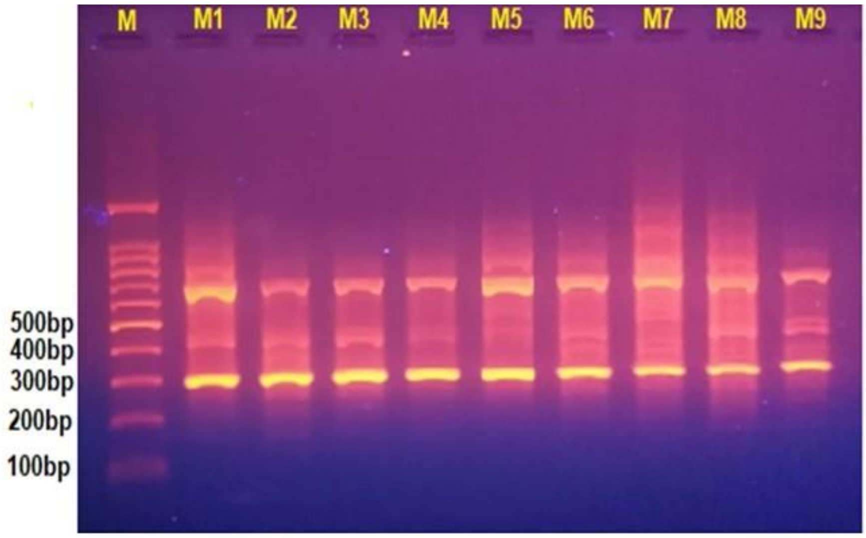

Plate 7. Gel electrophoresis of band pattern obtained from HB13 primer with collected samples of *Populus* species surveyed at four governorates of Egypt.

Abbreviations: M1: *P. euramericana* female Giza, M2: *P. euramericana* male Giza, M3: *P. deltoides* Giza, M4: *P. nigra* Cairo, M5: *P. nigra* Gharbia, M6: *P. deltoides* Gharbia, M7: *P. alba* Gharbia, M8: *P. euramericana* female Gharbia, and M9: *P. nigra* Qaliobia.

Table 6. Data analysis of band pattern of HB13 primer applied to nine samples of *Populus* plant species understudied at four governorates of Egypt.

Band no.	Mwt. (bp)	M1	M2	M3	M4	M5	M6	M7	M8	M9	Band type
1	985.573	0	0	0	0	0	0	1	0	0	U
2	936.696	0	0	0	0	0	0	1	0	0	U
3	789.388	0	1	0	0	1	1	0	0	0	P
4	709.410	1	1	0	0	0	1	0	0	0	P
5	559.367	1	1	1	1	1	1	1	1	1	M
6	486.246	0	0	0	0	1	0	0	0	0	U
7	434.912	0	0	0	0	0	0	1	0	1	P
8	330.193	1	1	1	1	1	1	1	1	1	M
9	200.000	1	1	1	1	1	1	1	1	1	M
Total no. of band for each sample		**4**	**5**	**3**	**3**	**5**	**5**	**6**	**3**	**4**	
Polymorphism%		**66.67%**									

Abbreviations: M1: *P. euramericana* female Giza, M2: *P. euramericana* male Giza, M3: *P. deltoides* Giza, M4: *P. nigra* Cairo, M5: *P. nigra* Gharbia, M6: *P. deltoides* Gharbia, M7: *P. alba* Gharbia, M8: *P. euramericana* female Gharbia, and M9: *P. nigra* Qaliobia.

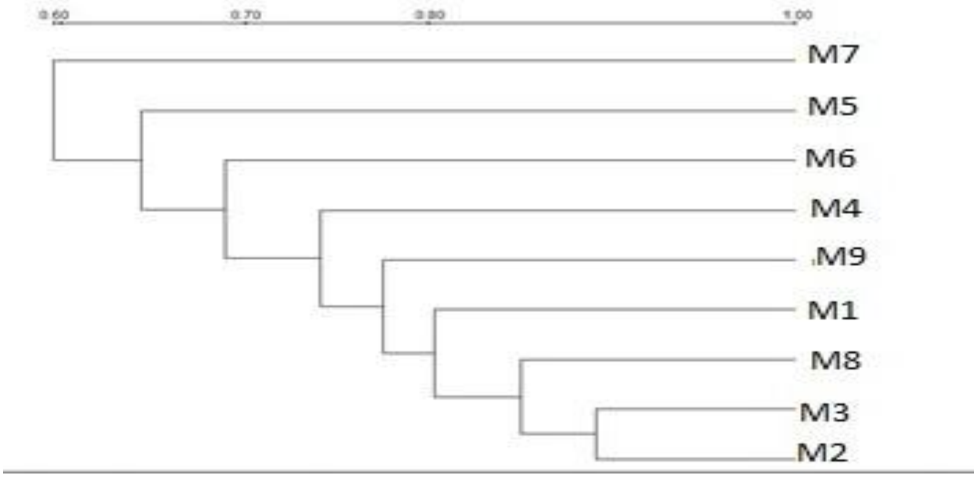

Plate 10. Dendogram for the differences among *Populus* species data surveyed at four governerates of Egypt according to the use of HB13 primer.
Abbreviations: M1: *P. euramericana* female Giza, M2: *P. euramericana* male Giza, M3: *P. deltoides* Giza, M4: *P. nigra* Cairo, M5: *P. nigra* Gharbia, M6: *P. deltoides* Gharbia, M7: *P. alba* Gharbia, M8: *P. euramericana* female Gharbia, and M9: *P. nigra* Qaliobia.

Table 7. List of primers name (ID), their nucleotide sequences and polymorphism percentage used in the ISSR analysis of nine different treatments of Populus subjected to genetic analysis.

No	Primer name	***Populus* treatments**		
		Total bands	**Polymorphic bands**	**Polymorphism %**
1	17899A	13	9	69.23
2	17899B	12	8	66.67
3	HB13	9	6	66.67
Total		**34**	**21**	**67.52**

Table 8. Total similarity matrix amongst nine different treatments of *Populus* subjected to genetic discrimination analysis.

Samples	M1	M2	M3	M4	M5	M6	M7	M8	M9
M1	100	66.7	71.8	62.5	53.0	57.3	46.3	72.3	55.8
M2	66.7	100	64.4	55.3	57.1	61.2	43.2	65.0	55.7
M3	71.8	64.4	100	64.7	51.4	55.3	42.6	64.4	56.0
M4	62.5	55.3	64.7	100	50.9	53.8	45.9	59.6	50.7
M5	53.0	57.1	51.4	50.9	100	61.3	34.2	56.3	56.4
M6	57.3	61.2	55.3	53.8	61.3	100	47.0	62.4	66.3
M7	46.3	43.2	42.6	45.9	34.2	47.0	100	46.9	45.5
M8	72.3	65.0	64.4	59.6	56.3	62.4	46.9	100	66.3
M9	55.8	55.7	56.0	50.7	56.4	66.3	45.5	66.3	100

Abbreviations: M1: *P. euramericana* female Giza, M2: *P. euramericana* male Giza, M3: *P. deltoides* Giza, M4: *P. nigra* Cairo, M5: *P. nigra* Gharbia, M6: *P. deltoides* Gharbia, M7: *P. alba* Gharbia, M8: *P. euramericana* female Gharbia, and M9: *P. nigra* Qaliobia.

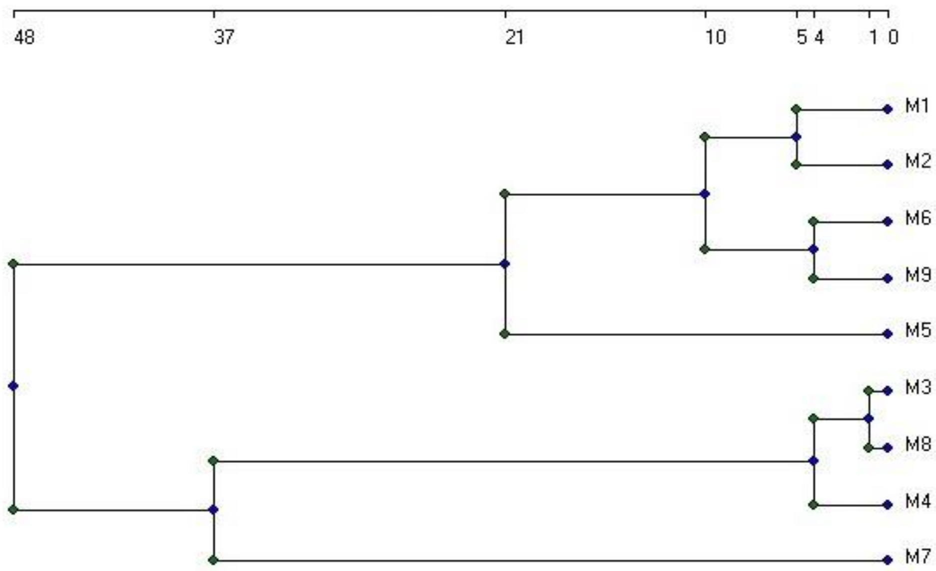

Plate 9. Neighbor-joining clustering trees constructed from amplified fragment length polymorphism genetic using three different primers data of field collection of *Populus* species.
Abbreviations: M1: *P. euramericana* female Giza, M2: *P. euramericana* male Giza, M3: *P. deltoides* Giza, M4: *P. nigra* Cairo, M5: *P. nigra* Gharbia, M6: *P. deltoides* Gharbia, M7: *P. alba* Gharbia, M8: *P. euramericana* female Gharbia, and M9: *P. nigra* Qaliobia.

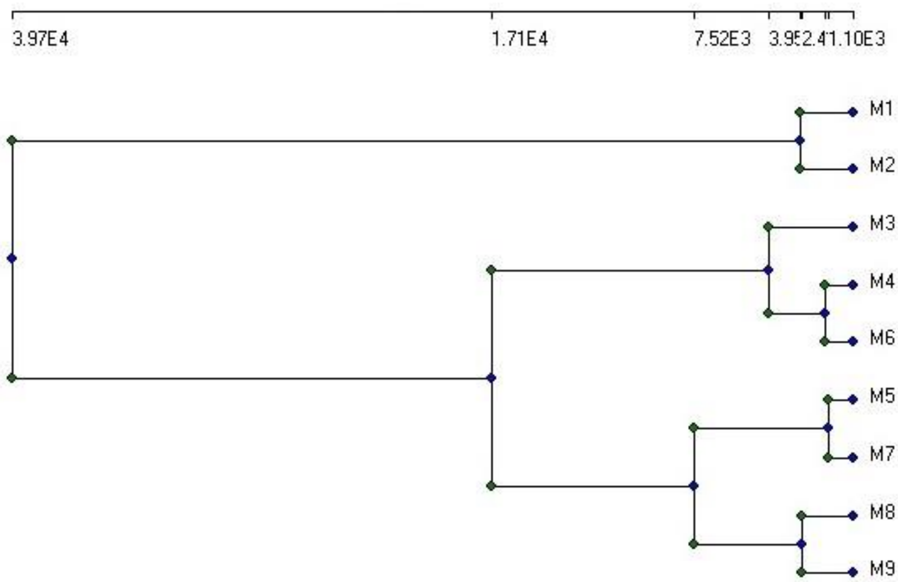

Plate 10. Neighbor-joining clustering trees constructed from morphological and amplified fragment length polymorphism genetic data of field collection of *Populus* species.
Abbreviations: M1: *P. euramericana* female Giza, M2: *P. euramericana* male Giza, M3: *P. deltoides* Giza, M4: *P. nigra* Cairo, M5: *P. nigra* Gharbia, M6: *P. deltoides* Gharbia, M7: *P. alba* Gharbia, M8: *P. euramericana* female Gharbia, and M9: *P. nigra* Qaliobia.

DISCUSSION

The survey trial in this research study was restricted to types of *Populus*, i.e. identifying species only and not to count up numbers of trees in the four Governorates involved, namely Giza, Cairo, Qaliobia and Gharbia. Survey data assembled was only restricted to information from research stations belonging to ARC (Ministry of Agriculture) located inside the four governorates. In Cairo and Qaliobia governorates, *P. nigra* was the only existed species. Whereas, in Giza, three species were found, viz. *P. euramericana* female, *P. euramericana* male and *P. deltoides*. Meanwhile, *P. nigra*, *P. deltoides*, *P. alba* and *P. euramericana* female existed in Gharbia governorate.

Exotic plants like maple trees, were found to introduce new morphological and biometric adaptation when they inhibit new sites (**Lamarque et al 2015)**). In this research study with *Populus*, this proved absolutely valid when DBH is used to discriminate among species, *P. deltoides* and *P. euramericana* female Giza and *P. deltoides* from Gharbia gave the largest DBH with noticed lower values were obtained from *P. euramericana* female in Gharbia and *P. nigra* in Qaliobia. Beside number of branches for *P. euramericana* female from Giza and *P. nigra* from Cairo demonstrated higher number. Whereas, *P. euramericana* female showed the lowest number of branches (3 branches).

All introduced *Populus* species showed high adaptation level to Egyptian territory across 4 different governorates. These findings suggest that phenotypic plasticity and genetic differentiation act synergistically to provide introduced tree populations substantial potential for rapid adaptation to new environmental conditions. Similar result was obtained by **Lamarque et al (2015)** showing the ability of exotic plants for adaptation. Thus, Egyptian environment is proper for woody species as well as some native woody plants from the flora.

LA plays an important aspect in research with regards to plant physiology in agriculture and dendrology (**Broadhead et al 2003**). Specifically, LA is involved in photosynthesis, transpiration, light interception, biomass estimation and water balance (**Kucharik et al 1998**). Leaf structure, LA and leaf shape vary tremendously between and within species as well as in response to environmental conditions, and these variations are known to play a key role in shaping plant functioning (**Nicotra et al 2011, Niinemets 2015**).

Results obtained here showed a variation amongst *Populus* species surveyed, both female and male of *P. euramericana* from Giza were superior in their LA. Whereas, the smallest LA was found in *P. alba* from Gharbia. Also, *P. nigra* was variate among surveyed sites where there was a significant difference between LA values. The huge increase of variation observed for LA indicates that the contrast among *Populus* species. The plant physiologists, biologists and agronomists have demonstrated the importance of LA in the growth analysis, the estimation of potential biological and agronomic yield, basis of the efficient use of solar radiation and mineral nutrition (**Sonnentag et al 2007**). Nevertheless, **Guet et al (2015)** showed within *P. nigra* nine population presence of various substantial variation and leaf trait plasticity. Leaf nitrogen showed the greatest divergence among populus population and previously found by **Bradshaw and Stettler (1995)**.

Significant Genotype × Environment (G × E) under variable soil water availability and temperature has also been documented, with examples in the genera *Eucalyptus* (**Dutkowski and Potts 2012**), *Pinus* (**Cregg and Zhang, 2001**), and *Populus* (**Cochard et al 2007**), *Populus nigra* **Fabbrini et al (2012)**. Similarly here, variations in morphological traits were found within *P. nigra* and *P. euramericana* in various site as well as among all species.

Results of ISSR patterns scored eight unique bands distinguishable species-specific bands for poplar, which could be used as molecular markers for poplar cultivated in Egypt. Several authors reported on the usefulness of ISSR for cultivar or species identifications. The similarity coefficient values and the UPGMA dendrogram revealed narrow genetic base among the tested species. This is likely due to the fact that their parental breeding lines used to develop these species were the same or were very close to each other. Expected diversity can be found even at small scale forest plantation as well as in our result of inhabitant *Populus* species. Similarly, variations in genetic traits were found within each of *P. nigra* and *P. euramericana* in sites surveyed. The consistent clustering derived from both phenotypic and genotypic data herewith proves the high diversity among surveyed *Populus* species.

Our research study suggest that *Populus* species possess genetic variation and can adapt to new environmental conditions, which in its self-valuable information would potentially lead to promising applications. Further assessment may be required on the flowering behaviors, leaf fall and flushing periodicity which could be tried on various poplar species that exist in Egyptian territories. Furthermore, tolerance of species and control to abiotic and biotic stress should be monitored under Egyptian territory condition.

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مجلد(27)، عدد(4)، 2273-2290، 2019
Website: http://ajs.journals.ekb.eg

التفرقه بين خمس انواع من الحور من حيث الصفات الظاهريه والكيميائية والتحليل الوراثي المنزرعة في اربع محافظات من مصر

[181]

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Received 7 October, 2019 Accepted 12 November, 2019

الموجـــــــز

أجريت الدراسة من خلال عمل حصر بمحطات البحوث التابعة لمركز البحوث الزراعية في أربع محافظات. استهدفت الدراسة حصر لأنواع الحور المنزرعة في أربع محافظات داخل جمهورية مصر العربية (الجيزة-القاهرة-القليوبية-الغربية)، والتفرقة بين أنواع الحور المختلفة المنزرعة على أساس الإختلافات في الصفات المورفولوجيـة والتركيب الوراثـي. نتج عن الحصر التعـــرف على الأنـــــواع التالية: *P. euramericana* female بمحافظة الجيزة والغربية و *P. euramericana* male بمحافظة الجيزة و *P. deltoides* بمحافظة الجيزة والغربيـة و *P. nigra* بمحافظة القاهـرة والغربيـة والقليوبية و *P. alba* بمحافظة الغربية. ونتج عن التوصيف المورفولوجي امكانيه التفرقه بين الانواع المختلفة عن طريق مساحة سطح الورقة ومتوسط الوزن الطازج والجاف للأوراق مكتملة النمو. تم كشف التباين الوراثي بين الأنواع المختلفة ونتج عنه نسبة إختلافات تصل الي 67.52% بين الأنواع التي تم حصرها من المحافظات المختلفة.

الكلمات الدالة: مصر، حصر، أشجار خشبية، الحور، الصفات الظاهرية، التحليل الوراثي، التتابع الداخلى البسيط

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