

Genetic diversity of *Typha domingensis* Pers. (Poales Typhaceae) and *Phragmites australis* (Cav.) Stued (Poales Poaceae) populations in lake Manzala coast and inland salines at Suez Canal region (Egypt) in relation to some ecological variables

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ABSTRACT

Typha domingensis Pers. (Poales Typhaceae) and *Phragmites australis* (Cav.) Stued (Poales Poaceae) are important wetland plants, valuable in remediation of wetland environment from heavy metals; moreover they can be used in biofuel production. Determination of genetic diversity in their natural populations is important for species conservation and ecological restoration. The present study compared the genetic variability of four populations of *T. domingensis* and *P. australis* growing in Manzala lake coast and inland swamps in Ismailia and Sinai by using random amplified polymorphic DNA (RAPD) technique. Nine primers generated a total of 175 RAPD bands (loci) of which 127 (72.57%) were polymorphic across all individuals of the two species. At Manzala lake coast (i.e. sites 3 and 4, contaminated sites), the genetic diversity measures (PPL%, I, h, N_a , N_e) observed in the populations of the two species showed higher diversity in comparison to the less contaminated sites 1 and 2 (Ismailia and Sinai). Gene diversity within populations (h_s) and total gene diversity (h_T) at species level were lower in *P. australis* (0.0104, 0.0579) than in *T. domingensis* (0.0825, 0.1284). This study revealed also the presence of a significant correlation between genetic diversity measures of *T. domingensis* and *P. australis* with some edaphic variables and heavy metal concentration in soil of the studied sites and leaves of the two species. The previous correlation indicated that populations from sites 3 and 4 respond with increased genetic variation, resulting possibly from new mutations affecting allele frequencies, as a consequence of adaptation to changes or disturbances in the environment. This may indicate that increased diversity levels may act as a buffer to severe heavy metal stress, which explains the importance of monitoring the genetic diversity of *T. domingensis* and *P. australis* populations in detecting trends that should alert ecologists to potential problems.

KEY WORDS

Genetic diversity; RAPD; environmental factors; Manzala; Sinai.

Received 08.11.2015; accepted 01.12.2015; printed 30.12.2015

INTRODUCTION

Typha domingensis Pers. and *Phragmites australis* (Cav.) Stued species form a major component

of wetland ecosystems in many parts of the world, including littoral zones of lakes, along rivers and irrigation/drainage canals, shallow fresh water swamps and anthropogenic habitats where soil is

periodically flooded (road side ditches, fields, stormwater retention basin). The geographical distribution of the two species extends from cold temperate regions to the tropics (Good, 1974; den Hartog et al., 1989).

In Egypt, *T. domingensis* and *P. australis* species are usually distributed throughout the running water of the main River Nile streams and its branches, irrigation and drainage canals as well as in the still water of some specific habitats like fresh water swamps and salt marshes (Serag et al., 1999). The two species are used by farmers (in Egypt and many other parts of the world) from ancient periods, for roofing, fencing and baskets manufacture. Ecologically, wetland plants are important for oxygen production, nutrient cycling, control of water quality, sediment stabilization and shelter for aquatic organisms and wildlife (Mohan & Hosetti, 1999).

Wetland plants are potentially studied as "biomonitors" that accumulate contaminants in their tissues and therefore may be analyzed to identify the abundances and bioavailability of such contaminants in aquatic environments. Therefore, the use of *T. domingensis* and *P. australis* appears to be particularly promising as they can accumulate heavy metals from sediments and water (Zurayk et al., 2001). Recently, Abideen et al. (2011) explored wetlands for its potential as lignocellulosic biomass of "good" quality for bioethanol production.

The main threats facing the management of wetland plants are due to the fact that its habitats are subjected to greater stress from various human activities. As a result, large quantities of organic and inorganic materials were introduced into these ecosystems (Zurayk et al., 2001). Understanding the effects of the environmental contaminants on the plant genome is crucial for preserving the evolutionary potential of natural populations, as genetic diversity provides potential to adapt to environmental changes (Bourret et al., 2008). Many chemical contaminants have been demonstrated to induce genetic mutations and therefore affect the genetic structure of populations (Hoffmann & Willi, 2008). The toxicity of different pollutants and their physical disturbance can influence plant survival, recruitment, reproductive success, mutation rates, and even migration and consequently affect the genetic diversity of exposed populations (Deng et al., 2007).

In the last few years, the field of molecular biology has provided new tools for studying population

structure and genetic diversity in wetland species. For example, cattain (*Typha* L.) and cordgrass (*Spartina* Schreb.) were studied for the first time, using allozyme polymorphism (McNaughton 1975; Silander, 1985; Raybould et al., 1991). Since the 1980s new perspectives in how to study population dynamics in common reed became available with the development of molecular markers (Jackson et al., 1985; de Kroon & van Groenendael, 1997). One of the most efficient molecular marker methods in terms of ability to produce polymorphic markers within a comparatively short time and with a limited budget is RAPD (random amplified polymorphic DNA). Since its introduction by Williams et al. (1990), RAPD has become widely used in various areas of plant research. Therefore, the aims of the present study are to: 1) determine habitat characteristics, 2) assess heavy metals accumulation in the leaves of *T. domingensis* and *P. australis*; and 3) describe these plants genetics using random amplified polymorphic DNA (RAPD), focusing on their genetic diversity and genetic differentiation. This study provides some molecular information to understand the genetic background to support the formulation of effective measures for genetic resources characterization, genetic improvement and sustainable utilization of these species.

MATERIAL AND METHODS

Plant populations

A total of 40 accessions of *T. domingensis* and 40 accessions of *P. australis* were used in this study. Soil samples and leaves were collected from four populations (see Table 1), namely two populations in the Manzala coastal land (indicated as Manzala lake 1, elgameel and Manzala lake 2, Bahr kuwar), one from the saline in Ismailia (gate of the industrial zone) and one from salt marshes in Sinai, at the east bank of Suez Canal (New Meet Abu elkom village).

Soil analysis

Three soil samples were collected from each stand at a depth of 0-50 cm, mixed, air-dried and passed through a 2-mm sieve for physical and chemical analyses. Soil texture was determined by the

use of Bouyoucos hydrometer; organic matter content was determined by Walkely and Black rapid titration method. Calcium carbonate content was estimated in the dry soil samples using Collins Calci-meter. Soil-water extracts (1:5) were used for the estimation of soil salinity (EC) using conductivity meter, soil reaction (pH) was determined using pH-meter, soluble carbonates (CO_3^{--}) and bicarbonates (HCO_3^-) by titration against standard H_2SO_4 using methyl orange and phenol- phenolphthalein as indicators, chlorides (Cl^-) by direct titration against standard AgNO_3 solution using K_2CrO_4 as an indicator, calcium and magnesium were estimated by Versene (EDTA) method. Sodium and potassium were determined using a flame photometer. All these procedures were according to Chapman & Pratt (1961), Jackson (1973), Allen et al. (1974), and Baruah & Barthakur (1997).

Heavy metals (Cd, Cr, Mn, Ni, Pb, Co, Cu and Fe) in soil samples were analyzed by the total sorbed metals method according to USEPA (1986) using atomic spectrophotometer. Leaves of *Typha domingensis* and *Phragmites australis* were collected at the four sites for heavy metals (Cd, Cr, Mn, Ni, Pb, Co, Cu and Fe) analysis using Perkin Elmer Atomic Absorption Spectrophotometer (model PYEUNICAM SP9, England) according to Allen et al. (1974). Soil characteristics supporting the four study populations and heavy metal measurements in leaves are shown in Tables 2 and 3.

DNA analysis

Fresh leaves of plants were collected and total genomic DNA was extracted using Wizard genomic DNA extraction kit promega (USA). 10- to 21-mer arbitrary primers were used for RAPD analysis.

Nine primers were screened for their amplification (Table 4). PCR amplification was performed in total volume of 25 μl containing 10 \times reaction buffer, 2.5 mM dNTPs, 5 mM MgCl_2 , 10 pmol/reaction primer, 100 ng of genomic DNA and (0.5 U/ μl) of Taq polymerase (promega, Germany) in Thermocycler Gene Amp 9700 (Applied Biosystems (ABI), USA). After a denaturation step for 5 min at 95°C, amplification reactions were carried out for 40 cycles. Each cycle comprised of 1 min at 95°C, 1 min of annealing temperature ranging from 28 to 30°C and 1 min at 72°C. The final elongation step was extended to 10 min.

Population	Population site	Longitude (N)	Latitude (E)	Elevation (m)
1	Industrial zone, Ismailia	30°34' 20.43"	32°11' 51.04"	15.41
2	New Meet about elkom, Sinai	30° 23' 57"	32°26 ' 6.28"	13.45
3	Manzala lake 1, elgameel	31°17' 12.42"	32°12' 42.79"	9.92
4	Manzala lake 2, Bahr kuwar	31°15' 43.57"	32°13' 12.16"	8.69

Table 1. Location of the collection sites of the four study populations of *Typha domingensis* and *Phragmites australis* and their respective geographic coordinates in Egypt.

Soil factor	(1) Elelwi bridge Ismailia	(2) New Meet Abu elkom village	(3) Manzala lake, elgameel	(4) Manzala lake 2, Bahr kuwar
Sand (%)	81	77	83	83
Silt (%)	2.6	4.6	2.6	2.6
Clay(%)	16.4	18.4	14.4	14.4
pH	10.3	9.52	8.71	8.55
CaCO_3 (%)	4.34	2.6	6.08	4.34
CO_3^{--} (ppm)	26.4	4.8	-	-
HCO_3^- (ppm)	2.44	21.96	29.28	12.2
O.M. (%)	0.68	0.37	0.71	0.37
EC (ms/cm)	5.29	22.3	2.56	1.96
Cl^- (ppm)	798.8	816.5	106.5	30
Ca^{++} (mg/100 gm)	390	330	370	500
Mg^{++} (mg/100 g)	44.6	39.6	32	17.6
Na^+ (mg/100 gm)	390	670	120	50
K^+ (mg/100 gm)	14.4	17.2	15.6	15.6
P (mg/100gm)	2.3	1.2	1.5	2.5
Fe (ppm)	23.2	9.7	21.1	19.4
Zn (ppm)	14	5	5.9	9.6
Ni (ppm)	18.7	15.4	8.8	5.5
Pb (ppm)	24	34	20	26
Cd (ppm)	0.07	0.28	0.35	0.42
Co (ppm)	0.9	3	1.8	4.2

Table 2. Characteristics of soil supporting the studied populations of *Typha domingensis* and *Phragmites australis*.

	SITE 1		SITE 2		SITE 3		SITE 4	
Metal conc	<i>P. aus</i>	<i>T. dom</i>						
Ni (ppm)	22	27.5	44	38.5	49.5	88	55	71.5
Pb (ppm)	90	60	130	70	80	90	40	10
Cd (ppm)	6.7	4.9	7	6.3	6.7	6	5.6	7.6
Co (ppm)	1.5	1.5	0	0	1.5	15	13.5	22.5
Fe (ppm)	194.4	183.6	156.6	135	200	405	43.2	283.5
Zn (ppm)	30.6	32.9	26.6	23.9	21.6	30.9	29.7	20.3

Table 3. Heavy metal concentration in the leaves of *Typha domingensis* and *Phragmites australis*.

Amplification products were separated on agarose gel electrophoresis using 1.5% (w/v) agarose in 0.5× TBE buffer, stained with ethidium bromide and photographed by using gel documentation system. Amplification products were analysed by a 100 to 1000 bp molecular weight marker.

Statistical analysis

RAPD bands were scored as binary presence (1) or absence (0) characters to assemble the matrix of the RAPD data. Then, the indices of genetic diversity, such as percentage of polymorphic loci (PPL), observed number of alleles (N_a), number of effective alleles (N_e), Nei's gene diversity (h), Shannon information index (I), the coefficient for gene divergence (G_{st}) and gene flow (N_m), and Hierarchical analysis of molecular variance (AMOVA) within and among populations were estimated using allele frequencies, by POPGENE 3.2 software (Yeh et al., 1999) GenAlEx version 6.4 (Peakall & Smouse, 2006).

The Pearson correlation between the genetic diversity index within population and ecological factors was analyzed using the SPSS 17 software.

RESULTS

Nine primers produced a total of 175 RAPD bands (loci), among which 127 were polymorphic. The number of bands per primer varied

from 5 to 39 with an average of 19.44. The average proportion of polymorphic markers across primers was 72.57%, ranging between 53.85% (UBC76) and 100% (UBC1) (Table 4), and these primers produced fragments ranging from 142 to 2066 bp in size.

The genetic diversity parameters (PPL%, I , h , N_a , N_e) among populations of *T. domingensis* showed higher values than *P. australis* at mean population level. In *T. domingensis*, PPL= 17.285%, I = 0.116, h = 0.082, N_a = 0.346, N_e = 1.159, respectively. For *P. australis*, the means of genetic parameters were PPL= 7.285%, I = 0.048, h = 0.034, N_a = 0.146, N_e = 1.065, respectively.

It was found that the genetic parameters in the population of *T. domingensis* growing in Manzala lake elgameel reached the highest values (PPL = 19.43%, I = 0.129, h = 0.091, N_a = 0.389, N_e =1.173) whereas in the population of *P. australis* growing in New Meet abou elkom, Sinai, attained the lowest (PPL = 2.86%, I = 0.018, h = 0.010, N_a = 0.057, N_e =1.022) (Table 5).

Gene diversity within populations (h_s) and total gene diversity (h_T) at species level, were lower in *P. australis* (0.0104, 0.0579) compared to *T. domingensis* (0.0825, 0.1284). Low values of G_{st} were estimated at species level for *T. domingensis* (0.36) and *P. australis* (0.42). The estimate of gene flow N_m based on G_{st} for *T. domingensis* and *P. australis* populations was 0.8985 and 0.6946, respectively, which indicated that gene flow among populations was low (Table 6).

Soil and heavy metals analysis

Soil chemical and physical features are in Table 2. As shown, soil of site 1 (Industrial zone, Ismailia) had the highest values of pH (10.3), CO₃ (26.4 ppm), Mg⁺⁺ (44.6 mg/100 gm soil) and the lowest values of HCO₃⁻ (2.44 ppm). Soil of site 2 (New

Meet about elkom, Sinai) attained the highest values of silt (4.5%), clay (18.4%), EC (22.3 mm/cm, CL (816.5 ppm), Na⁺ (670 mg/100 mg soil and K⁺ (17.2 mg/100 mg soil) but the lowest of sand (77%), CaCO₃ (2.6%) and Ca⁺⁺ (330 mg/100 gm soil). Soil of site 3 (Manzala lake, elgameel) showed the highest values of sand (83%), CaCO₃ (6.08%),

Primer	Sequences of primer (5→3)	Total number of bands	Number of polymorphic bands	Percent of polymorphic bands %
UBC1	CCTTCGGCTC	5	5	100
UBC3	GGCTTGACCT	7	5	71.34
UBC6	GAAGGCGAGA	8	5	62.5
UBC9	GTCATGCGAC	16	15	93.75
UBC13	CCTGGCACAG	17	15	88.24
UBC16	CCAGACTCCA	30	22	73.33
UBC64	GAGGGCGGGA	39	30	76.92
UBC76	GAGCACCAGT	26	14	53.85
UBC77	GAGCACCAGG	27	16	59.26
	Total	175	127	
	Average	19.44	13.89	72.57

Table 4. Sequences of the nine primers used in this study.

Pop.	Species	Sample size	Polymorphic loci	Percentage population level (PPL%)	Observed number of alleles N _a	Number of effective alleles N _e	Shannon's index of diversity (I)	Nei's gene diversity (h)
1	<i>T. domingensis</i>	10	30	17.14	0.343	1.166	0.117	0.084
2	<i>T. domingensis</i>	10	26	14.86	0.297	1.137	0.100	0.070
3	<i>T. domingensis</i>	10	34	19.43	0.389	1.173	0.129	0.091
4	<i>T. domingensis</i>	10	31	17.71	0.354	1.161	0.118	0.084
	Mean	10	30.25	17.285	0.346	1.159	0.116	0.082
1	<i>P. australis</i>	10	10	5.71	0.114	1.052	0.038	0.027
2	<i>P. australis</i>	10	4	2.86	0.057	1.022	0.018	0.010
3	<i>P. australis</i>	10	23	13.14	0.263	1.126	0.090	0.064
4	<i>P. australis</i>	10	13	7.43	0.149	1.060	0.047	0.033
	Mean	10	12.5	7.285	0.146	1.065	0.048	0.034
	Overall mean		21.375	12.29	0.246	1.112	0.082	0.058

Table 5. Genetic diversity parameters in plant populations of *Typha domingensis* and *Phragmites australis*.

HCO_3^- (29.28) and O.M. (0.71%) and the lowest of pH (8.71). Soils of Manzala lake 2, Bahr kuwar had the highest values of Ca^{++} (500 mg/100 mg soil) and the lowest of Cl^- (30 ppm), Na^+ (50 mg/ 100 mg soil) and Mg^{++} (17.6 mg/100 mg soil).

Heavy metals (Iron, Zinc, Nickel, Lead, Cadmium and Cobalt) were recorded in high concentrations in all studied sites. The highest values of Iron and Zinc (23.2 ppm and 14 ppm) were recorded in site 1. Lead recorded the highest value (34 ppm) in site 2. The highest values of Cadmium (0.42 ppm) and Cobalt (4.2 ppm) were recorded in Site 4.

The estimate of heavy metals content in the leaves of *P. australis* and *T. domingensis* indicated the highest accumulation in the leaves of *T. domingensis*, Zn (32.9 ppm) in site 1, Ni (88 ppm) and Fe (405 ppm) in site 3, Cd (7.6 ppm) and Co (22.5 ppm) in site 4. On the other hand, *P. australis* showed the highest accumulation of Pb (130 ppm) in site 2 (Table 3).

Some combinations of soil and genetic diversity indices of *T. domingensis* and *P. australis* produced significant positive correlations, such as sand, CaCO_3 and O.M. with PPL%, N_a , N_e , h and I of the two species. On the other hand, Silt, Clay, pH, EC, Cl^- , Na^+ and K^+ produced significant negative correlations with PPL%, N_a , N_e , h and I of the two species also. Ca^{++} showed significant positive correlations with PPL%, ($r = 0.36$), N_a ($r = 0.36$), N_e ($r = 0.38$), h ($r = 0.39$) and I ($r = 0.38$) of *T. domingensis*. Similarly, HCO_3^- produced significant positive correlations with PPL% ($r = 0.48$), N_a ($r = 0.48$), N_e ($r = 0.49$), h ($r = 0.45$) and I ($r = 0.49$) of *P. australis*.

Population group	h_s	h_T	N_m	G_{st}
<i>T. domingensis</i>	0.0825	0.1284	0.8985	0.3575
SD	0.0081	0.0174	-	-
<i>P. australis</i>	0.0104	0.0579	0.6946	0.4186
SD	0.0037	0.0104	-	-

Table 6. Genetic differentiation at species level of *Typha domingensis* and *Phragmites australis* study sites; h_s = Gene diversity within population, h_T = total gene diversity, N_m = estimate of gene flow, and G_{st} = coefficient of gene differentiation.

Correlations between some heavy metal concentrations in the soils of the studied sites and genetic diversity indices of *T. domingensis* and *P. australis* indicated that, Fe showed significant positive correlations with PPL%, N_a , N_e , h and I of the two species. On the other hand, Ni and Pb produced significant negative correlations with PPL%, N_a , N_e , h and I of the two species. Zn produced significant positive correlations with N_a ($r = 0.25$) of *T. domingensis* and Cd produced significant positive correlations with PPL% ($r = 0.39$), N_a ($r = 0.39$), h ($r = 0.36$) and I ($r = 0.36$) of *P. australis* (Table 7).

Correlations between some heavy metal concentrations in the leaves of *T. domingensis* and *P. australis* and genetic diversity parameters of both species showed that, Ni, Co and Fe produced significant positive correlations with PPL% ($r = 0.76$, 0.68 and 0.94 respectively), N_a ($r = 0.76$, 0.68 and 0.94 respectively), N_e ($r = 0.51$, 0.51 and 0.77), h ($r = 0.63$, 0.60 and 0.86 respectively) and I ($r = 0.68$, 0.63 and 0.89, respectively) of *T. domingensis*. Ni produced significant positive correlations with PPL% ($r = 0.36$), N_a ($r = 0.37$), and I ($r = 0.33$) of *P. australis*. On the other hand, Pb produced significant negative correlations with PPL% ($r = -0.51$), N_a ($r = -0.51$), N_e ($r = -0.43$), h ($r = -0.43$) and I ($r = -0.49$) of *P. australis*. Zinc demonstrated significant positive correlations with PPL% ($r = 0.36$), N_a ($r = 0.37$), N_e ($r = 0.53$), h ($r = 0.45$) and I ($r = 0.43$) of *T. domingensis* and significant negative correlations with PPL% ($r = -0.67$), N_a ($r = -0.67$), N_e ($r = -0.7$), h ($r = -0.66$) and I ($r = -0.68$) of *P. australis* (Table 8).

DISCUSSION

The genetic diversity in wetland plant populations has been reviewed in some studies, and a considerable amounts of diversity have been found in most plant species (Tsyusko et al., 2005; Diyanat et al., 2011). Studies on *P. australis* and *T. domingensis* examining genetic variation showed high levels of genetic differentiation among populations (Zeidler et al., 1994; Koppitz et al., 1997; McLellan et al., 1997).

RAPD is an effective method to detect intra- and interpopulation variation and is still used widely in many plants (Koppitz et al., 1997; Koppitz, 1999;

Soil variables	<i>Typha domingensis</i>					<i>Phragmites australis</i>				
	PPL%	N _a	N _e	H	I	PPL%	N _a	N _e	H	I
Sand	0.98**	0.93**	0.91**	0.94**	0.94**	0.81**	0.81**	0.76**	0.81**	0.79**
Silt	-0.86**	-0.86**	-0.95**	-0.93**	-0.91**	-0.68**	-0.68**	-0.65**	-0.70**	-0.67**
Clay	-0.92**	-0.92**	-0.85**	-0.90**	-0.91**	-0.84**	-0.84**	-0.79**	-0.87**	-0.81**
pH	-0.50**	-0.49**	-0.21	-0.35*	-0.40*	-0.58**	-0.58**	-0.52**	-0.54**	-0.55**
CaCO ₃	0.99**	0.99**	0.94**	0.97**	0.98**	0.97**	0.97**	0.97**	0.98**	0.97**
HCO ₃	0.22	0.21	-0.09	0.04	0.10	0.48**	0.48**	0.49**	0.45**	0.49**
OM	0.64**	0.64**	0.76**	0.71**	0.69**	0.62**	0.61**	0.68**	0.66**	0.64**
EC	-0.89**	-0.89**	-0.94**	-0.94**	-0.92**	-0.73**	-0.73**	-0.70**	-0.74**	-0.71**
Cl ⁻	-0.77**	-0.76**	-0.57**	-0.67**	-0.71**	-0.76**	-0.76**	-0.70**	-0.73**	-0.73**
Ca ⁺⁺	0.36*	0.36*	0.38*	0.39*	0.38*	0.17	0.17	0.08	0.15	0.13
Na ⁺	-0.88**	-0.88**	-0.80**	-0.86**	-0.86**	-0.78**	-0.78**	-0.72**	-0.77**	-0.75**
K ⁺	-0.57**	-0.57**	-0.81**	-0.72**	-0.67**	-0.35*	-0.35*	-0.35*	-0.38*	-0.35*
Fe	0.79**	0.79**	0.95**	0.89**	0.86**	0.60**	0.60**	0.60**	0.63**	0.60**
Zn	0.11	0.11	0.43**	0.29	0.23	-0.16	-0.16	-0.16	-0.12	-0.16
Ni	-0.55**	-0.55**	-0.31	-0.44**	-0.48**	-0.58**	-0.58**	-0.50**	-0.53**	-0.54**
Pb	-0.96**	-0.96**	-0.99**	-0.99**	-0.98**	-0.88**	-0.88**	-0.88**	-0.90**	-0.88**
Cd	0.28	0.28	-0.02	0.12	0.18	0.39*	0.39*	0.32	0.34*	0.36*

Table 7. Pearson correlation coefficient (r value) between the soil variables and genetic diversity parameters of *Typha domingensis* and *Phragmites australis*; ** correlation is significant at the 0.01 level (2-tailed); * correlation is significant at the 0.05 level (2-tailed).

Metal conc.	<i>Typha domingensis</i>					<i>Phragmites australis</i>				
	PPL%	N _a	N _e	H	I	PPL%	N _a	N _e	H	I
Ni	0.76**	0.76**	0.51**	0.63**	0.68**	0.36*	0.37*	0.30	0.31	0.34*
Pb	0.10	0.10	0.05	0.06	0.07	-0.51**	-0.51**	-0.43**	-0.49**	-0.47**
Cd	0.02	0.02	-0.21	-0.10	-0.06	-0.19	-0.19	-0.09	-0.16	-0.14
Co	0.68**	0.68**	0.51**	0.60**	0.63**	0.10	0.10	0.00	0.07	0.06
Fe	0.94**	0.94**	0.77**	0.86**	0.89**	0.19	0.19	0.29	0.23	0.23
Zn	0.36*	0.37*	0.53**	0.45**	0.43**	-0.67**	-0.67**	-0.70**	-0.66**	-0.68**

Table 8. Pearson correlation coefficient (r value) between the concentrations of heavy metal variables in leaves and genetic diversity parameters of *Typha domingensis* and *Phragmites australis*; ** correlation is significant at the 0.01 level (2-tailed); * correlation is significant at the 0.05 level (2-tailed).

Keller, 2000; Bussell et al., 2005; Curn et al., 2007). Our results also show that RAPD is suitable for genetic diversity assessment in *P. australis* and *T. domingensis*.

Attempts were made in this study to use environmental variations for appropriately interpreting genetic information of *P. australis* and *T. domingensis*. A number of previous studies have shown that there is a correlation between genetic diversity and environmental heterogeneity in common reed populations (Hargeby et al., 2004; Curn et al., 2007; Hansen et al., 2007; Engloner, 2009), but very few studies have explicitly tested the causal environmental factors behind the pattern of genetic variation. In our study we found significant positive correlations of sand, CaCO₃ and O.M. with all the genetic parameters of the two species and significant negative correlations of Silt, Clay, pH, EC, Cl⁻, Na⁺ and K⁺ with the two species. Heather et al. (2011) found significant negative correlations between genotypic richness of *P. australis* and potassium concentration in the soil. Similarly, Lexuan et al. (2012) found significant negative correlations between soil salinity and genetic diversity of *P. australis*.

Soil analyses revealed that the coastal sites of Manzala lake (site 3 and site 4) have higher levels of cadmium and cobalt whereas the sites of salines in industrial zone, Ismailia and new Meet Abou Elkoum, Sinai (site1 and site2) consistently grouped as the sites with the significantly least amount of metals. The present study showed significant positive correlation between genetic diversity parameters and some heavy metals such as Iron, Zinc and Cadmium and significant negative correlation between genetic diversity parameters with Nickel and Lead. The high level of genetic variability within *T. domingensis* and *P. australis* from site 3 and site 4 could be ascribed in part to these conditions. These findings are in conjunction with the results reported by Bush & Barret (1993) on isozyme diversity that indicate the population grown in contaminated sites were higher polymorphic than uncontaminated populations. Brian et al. (1999) detected that there are significantly higher genetic diversity at polluted sites. The retention of such elevated levels of genetic diversity within these contaminated populations can be attributed to a number of selective, reproductive and demographic factors. As described by Bourret et al.

(2007) if tolerance to the adverse environmental condition increases as a function of individual heterozygosity and/or if the contaminant is a mutagen, genetic variation within the affected population will remain elevated and may increase. The correspondence between ecological and genetic landscapes may be indicative of the potential role of environmental variables in driving population divergence (Schlotterer et al., 2004; Nielsen, 2005; Guo & Mrazek, 2008; Hancock et al., 2010). Possibly, these variations among studied populations will assist in successful management of *P. australis* and *T. domingensis*.

CONCLUSIONS

In conclusion, the present results demonstrated that both *T. domingensis* and *P. australis* showed high capacity of metal bioaccumulation, moreover higher genetic diversity is found in *T. domingensis*, especially in contaminated sites, than in *P. australis*. Overall, the correspondence between ecological and genetic landscapes may be indicative of the potential role of environmental variables in driving population differences (Schlotterer et al., 2004; Nielsen, 2005; Guo & Mrazek, 2008; Hancock et al., 2010).

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