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(RESEARCH ARTICLE)

Analysis of genetic diversity and bioaccumulation potential of *Juncus acutus* grown in some northern swamp habitats of Egypt

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Abstract

Juncus acutus is one of main wetland plants, valuable in remediation of wetland environment from heavy metals. Determination of genetic diversity in its natural populations is important for species conservation and ecological restoration. The present study evaluated the genetic diversity of four populations of *J. acutus* growing in Manzala lake coast and inland swamps in Ismailia and Sinai by using random amplified polymorphic DNA (RAPD) technique. Fifteen primers generated a total of 217 RAPD bands (loci) of which 156 (71.9%) were polymorphic across all individuals of the four populations. At Manzala lake coast (i.e. sites 3 and 4, contaminated sites), the genetic diversity measures observed in the populations of the two species showed higher diversity in comparison to the less contaminated sites 1 and 2 (Ismailia and Sinai). This study revealed also the presence of a noticeable accordance between genetic diversity measures of *J. acutus* with some edaphic variables and heavy metal concentration in soil of the studied sites and leaves of the two species and it indicated that populations from sites 3 and 4 respond with increased genetic variation, resulting possibly from new mutations affecting allele frequencies, as a consequences of adaptation to changes or disturbances in the environment. This may indicate that the increased diversity levels may act as a buffer to severe heavy metal stress, which explains the importance of monitoring the genetic diversity of *J. acutus* populations in detecting trends that should alert ecologists to potential problems.

Keywords: *Juncus acutus*; Manzala; Sinai; Genetic diversity; Pollution; Bioaccumulation

1. Introduction

Juncus acutus was considered as one of the major halophyte species of wetland ecosystems in whole over the world, including coastal zones of lakes, along rivers and irrigation/drainage canals, marshlands and anthropogenic habitats where soil is periodically flooded (road side ditches, fields, storm- water retention basin), its geographical distribution outspreads from cold temperate regions to the tropics [1-2].

Juncus acutus species are perennial grass like with wide distribution, it is autochthonous monocotyledons thriving in wetland. In Egypt it, 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 [3]. *J. acutus* is used by farmers (in Egypt and many other parts of the world) from ancient periods, for roofing, fencing, mats, and baskets manufacture. Ecologically, *J. acutus* is important for oxygen production - they release high amount of Oxygen in the rhizosphere-, nutrient cycling, control of water quality, sediment stabilization and shelter for aquatic organisms and wildlife [4].

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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 *helophyte species appears* to be particularly promising as they can accumulate heavy metals from sediments and water [5]. Along with metals accumulation in the below ground plant parts, phytostabilization has also been reported as another remediation mechanism in the root zone. In addition, recent literature has revealed the potential contribution of *Juncus* sp. on the removal of EDCs and PPCPs.

The main threats facing the management of *J. acutus* 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 [5]. 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 [6]. Many chemical contaminants have been demonstrated to induce genetic mutations and therefore affect the genetic structure of populations [7]. 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 [8].

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*) and cordgrass (*Spartina*) were studied for the first time, using allozyme polymorphism [9-11]. Since the 1980s new perspectives in how to study population dynamics in common reed became available with the development of molecular markers [12-13]. 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 [14], RAPD has become widely used in various areas of plant research.

Taking into account that the ability of plants to accumulate compounds may be affected by the variation and high levels of contaminants. Therefore, the aims of the present study are to: 1) determine habitat characteristics, 2) assess heavy metals accumulation in the plant parts of *J. acutus*; and 3) describe this plants genetics using random amplified polymorphic DNA (RAPD), focusing on their genetic diversity and genetic differentiation and determine the relations between gentic variation and heavy metals bioaccumulation of the existing sampled populattions of *J. acutus*. 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 *J. acutus*.

2. Material and methods

2.1. Plant populations

A total of 60 accessions of *J. acutus* 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).

Table 1 Location of the collection sites of the four study populations of *Juncus acutus* and their respective geographic coordinates in Egypt

Population	Population site	Longitude (N)	Latitude (E)	Elevation (m)
1	Industerial zone, Ismailia	30˚ 34 20.43"	32˚ 11 51.04"	15.41
2	New Meet abou elkom, Sinai	30˚ 23' 57"	32˚ 26 ' 36.28"	13.45
3	Elgameel, Manzala lake 1	31˚ 17' 12.42"	32˚12' 42.79"	9.92
4	Bahr kuwar, Manzala lake 2	31˚ 15' 43.57"	32˚ 13' 12.16"	8.69

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 Calcimeter. 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 [15- 18].

Heavy metals (Cd, Cr, Mn, Ni, Pb, Co, Cu and Fe) in soil samples were analyzed by the total sorbed metals method according to [19] using atomic spectrophotometer.

2.2. Plant analysis

Leaves of *J. acutus* 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 [17]. Soil characteristics supporting the four study populations and heavy metal measurements in leaves are shown in Tables 2 and 3.

2.3. 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.

Fifteen primers were screened for their amplification (Table 2). PCR amplification was performed in total volume of 25 µl containing 10× 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. 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.

Table 2 Sequences of the 15 primers used in this study

Primer	Sequences of primer (5→ 3)	Total number of bands	Number of polymorphic bands	Percent of polymorphic bands %
UBC1	CCTTCGGCTC	8	5	63
UBC3	GGCTTGACCT	5	3	60
UBC6	GAAGGCGAGA	10	7	70
UBC9	GTCATGCGAC	15	12	80
UBC13	CCTGGCACAG	18	13	72
UBC16	CCAGACTCCA	28	20	71
UBC64	GAGGGCGGGA	30	25	83
UBC76	GAGCACCAGT	25	17	68
UBC77	GAGCACCAGG	21	15	71
OPA02	TGCCGAGCTG	6	5	83
OPA04	AATCGGGCTG	4	2	50
OPA18	AGGTGACCGT	10	8	80
OPB01	GTTTCGCTCC	6	4	67
OPB05	TGCGCCCTTC	19	12	63
OPC08	TGGACCGGTG	12	8	58
	Total	217	156	71.9
	Average	15	10	73

2.4. 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), Shanon information index (I), the coefficient for gene divergence (G_{st}) were estimated using allele frequencies, by POPGENE 3.2 software [20] GenAlEx version 6.4 [21].

3. Results

Fifteen primers produced a total of 217 RAPD bands (loci), among which 156 were polymorphic. The number of bands per primer varied from 4 to 30 with an average of 15. The average proportion of polymorphic markers across primers was 69%, ranging between 50% (OPA04) and 83% (OPA02) (Table 2).

The genetic diversity parameters (PPL%, N_a, N_e, h, I) among populations of *J. acutus* showed that, the population no. (4) of *J. acutus* growing in Bahr Kwar site reached the highest values (PPL = 68.20%, N_a = 1.6820, N_e =1.472, h= 0.264, I = 0.387) whereas in the population no. (2) of *J. acutus* growing in new Meet abou elkom site, Sinai attained the lowest values (PPL= 44.24%, N_a = 1.442, N_e =1.472, h= 0.164, I = 0.242), Figure (1).

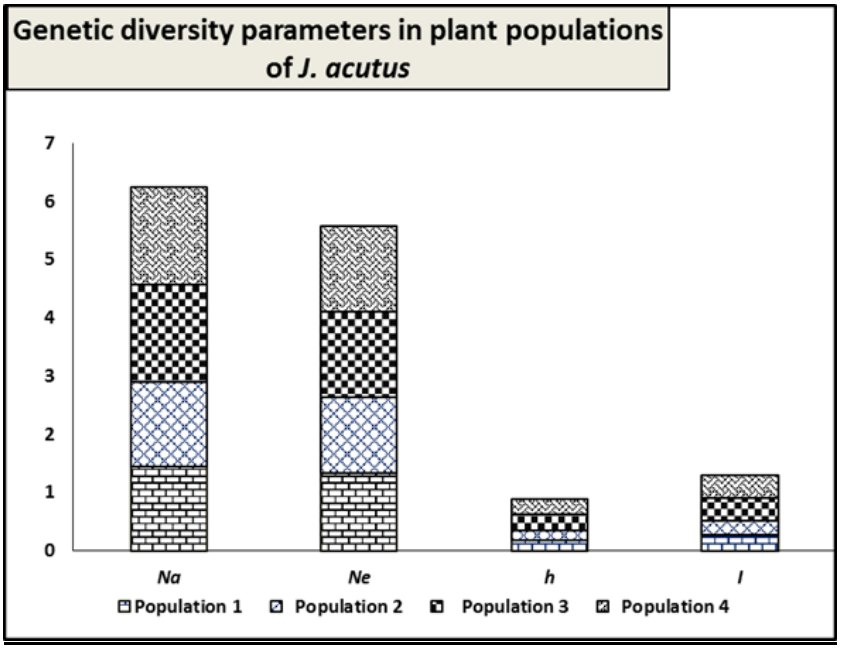

Figure 1 Genetic diversity parameters in plant populations of *J. acutus*: observed number of alleles (N_a), number of effective alleles (N_e), Nei's gene diversity (h), and Shanon information index (I)

Soil chemical and physical features are in Table 3. As shown, soil of site 1(Industrial zone, Ismailia) had the highest values of pH (10.25) , CO_3 (22.4 ppm), Mg^{++} (42.6mg/100 gm soil) and the lowest values of HCO_3^- (18.34 ppm). Soil of site 2 (Meet abou elkom, Sinai) attained the highest values of silt (4.8%), clay (18.2%), EC (23.2 mm/cm, CL (812.5 ppm), Na^+ (672 mg/100 mg soil and K^+ (16.2 mg/100 mg soil) but the lowest of sand (72%), $CaCO_3$ (2.8%) and Ca^{++} (331 mg/100 gm soil). Soil of site 3 (Manzala lake, elgameel) showed the highest values of sand (85%), $CaCo_3$ (5.88%), HCO_3^- (27.28) and O.M. (0.70%) and the lowest of pH (8.53). Soils of Site 4 (Manzala lake 2, Bahr kuwar) had the highest values of Ca^{++} (520 mg/100 mg soil) and the lowest of Cl^- (104 ppm), Na (48 mg/ 100 mg soil) and Mg (16.6 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 (22.2 ppm and 12.9 ppm) were recorded in site 1. Lead recorded the highest value (33 ppm) in site 2. The highest values of Cadmium (0.40 ppm) and Cobalt (4.4 ppm) were recorded in Site 4.

Table 3 The soil characteristics supporting the studied populations of *J. acutus*

Soil factor	Sites			
	(1)	(2)	(3)	(4)
Sand (%)	80	72	85	81
Silt (%)	2.5	4.8	2.4	2.4
Clay (%)	15.4	18.2	14.2	14.3
pH	10.25	9.33	8.53	8.66
$CaCO_3$ (%)	5.24	2.8	5.88	4.24
$CO3^{-}$(ppm)	22.4	4.75	2.25	2.22
HCO_3^- (ppm)	18.34	20.96	27.28	22.2
O.M. (%)	0.66	0.33	0.70	0.36
EC (ms/cm)	4.29	23.2	2.66	1.93
Cl^- (ppm)	790.8	812.5	300	104.5
Ca^{++} (mg/100 gm)	380	331	372	520
Mg^{++} (mg/100 g)	42.6	37.6	30	16.6
Na^+ (mg/100 gm)	382	672	119	48
K^+ (mg/100 gm)	12.4	16.2	15.5	14.6
P (mg/100gm	2.2	1.3	2.4	2.6
Fe (ppm)	22.2	9.5	21.2	19.5
Zn (ppm)	12.9	5.1	5.8	9.4
Ni (ppm)	18.6	15.2	8.6	5.4
Pb (ppm)	22	33	22	28
Cd (ppm)	0.08	0.24	0.33	0.40
CO (ppm)	0.95	2.9	1.9	4.4

The estimate of heavy metals content in the leaves of *J. acutus* indicated the highest accumulation of Zn (33.9 ppm) , Ni (48.5 ppm) in site 3 and Fe (188.1 ppm) in site 1, Cd (8.2 ppm), Co (12.2ppm), and Pb (91 ppm) in site 4. (Figure 2).

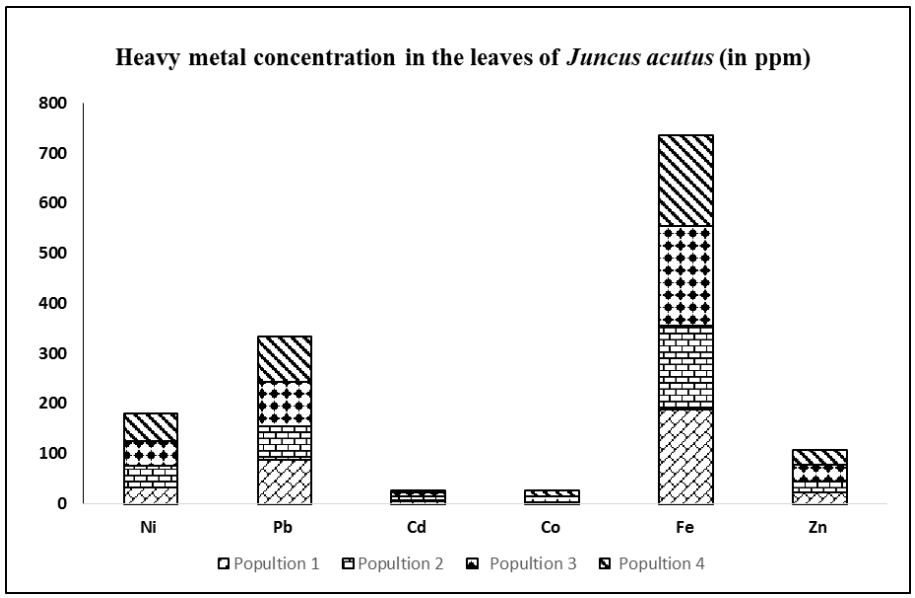

Figure 2 Heavy metal concentration in the leaves of *Juncus acutus* (in ppm)

4. 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 (22-23). Studies on *J. acutus* examining genetic variation showed high levels of genetic differentiation among populations [24- 26].

RAPD is an effective method to detect population variation and is still used widely in many plants [25, 27-30]. Our results also show that RAPD is suitable for genetic diversity assessment in *J. acutus.*

In our syudy we attempt to apply the data of environmental variations for appropriately interpreting genetic information of *J. acutus.* A number of previous studies have shown that there is a correlation between genetic diversity and environmental heterogeneity *J. acutus* and other related wet land species [31, 30, 32], but very few studies have explicitly tested the causal environmental factors behind the pattern of genetic variation.

In our study the values of the measurments of $CaCO_3$ and O.M. are in accordance with all the genetic parameters of the *J.acutus* populations. 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 Abu Elkoum, Sinai (site1 and site2) consistently grouped as the sites with the significantly least amount of metals.

The present study the genetic diversity parameters in parallel some heavy metals values such as Iron and Cadmium in sites 3 and 4. These findings are in conjunction with the results reported by [33] 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 [34]. 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 [6] 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 [35-36]. Possibly, these variations among studied populations will assist in successful management of *J. acutus.*

5. Conclusion

In conclusion, the present results demonstrated that *J. acutus* showed high capacity of metal bioaccumulation, moreover higher genetic diversity especially in contaminated sites in studied sites of Manzala lake. Overall, the correspondence between ecological and genetic landscapes may be indicative of the potential role of environmental variables in driving population differences.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The author have no conflict of interest (only one author for the manuscript).

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