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Effects of NaCl on growth, yield and ion concentration of various *Populus euphratica* Oliv. ecotypes in Iran

H. Tavakoli Neko[a*], A. Shirvany[b], M.H. Assareh[c], M.R. Naghavi[d], M. Pessarakli[e], A. Pourmeidani[a]

[a] *Forests and Rangelands Research Department, Qom Agricultural and Natural Resources Research and Education Center, AREEO, Qom, Iran*
[b] *Department of Forestry, Faculty of Natural Resources Sciences, University of Tehran, Karaj, Iran*
[c] *Research institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran*
[d] *Department of Agronomy and Plant breeding, Faculty of Agricultural Sciences, University of Tehran, Karaj, Iran*
[e] *School of Plant Sciences, the University of Arizona, Tucson, Arizona, USA*

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Abstract

Euphrates poplar (*Populus euphratica* Oliv.) is a woody species that is naturally distributed in the desert areas of some parts of Asia and Africa. Because of its outstanding features, it is a model plant to study environmental stress tolerance. This research was conducted from 2014 to 2016 in order to study the relationship between performance indices and ion concentrations. The cuttings of 12 ecotypes were collected from different climatic conditions in Iran. Salinity stress was applied using four levels of NaCl (75, 150, 225 and 300 mM) and one control sample (salt-free). The performance indices [diameter and height growth, biomass production; leaf, stem, root and total biomass] showed significant differences in salt levels and ecotypes. The ion concentrations showed significant differences in salt levels (except Ca^{2+}) and varied in different ecotypes. There was no significant difference in salt×ecotype interaction for most of the variables. The ecotypes, treatments, (salt levels) means of performance indices and ion concentrations were separated into different groups. Correlation coefficients showed that the concentration of macronutrients had positive correlations with performance indices, and that salt ions had negative correlations. Correlation coefficients also showed that the ion concentrations had synergistic or antagonistic effects on each other. The results of this study showed that the key mechanisms of salt tolerance in this specie include: exclusion of salt from the root, compartmentalization of Na^+ in plant tissue, preventing excessive reduction of K^+ absorption resulting in the maintenance of the K^+/Na^+ balance.

Keywords: Ions; *Populus euphratica*; Salt stress; Yield

1. Introduction

Salinity is an important abiotic stress affecting plant growth and productivity. Saline/sodic soils constitute about 26% of the world's cultivated lands. Usually, this threat mostly affects countries in developing and underdeveloped parts of Asia, Africa, and South America, which are in arid/semi-arid regions (Ansari *et al.*, 1999). Salt-affected soils can be observed in over 100 countries in varying form, nature, and properties. In general, studies are directed towards arid and semi-arid regions, even though no climatic zone is safe from salinization (Rengasamy, 2006). Different types of salinization with a prevalence of Na^+ salts affect about 34% of Iranian land (55.6 million hectares). About 4.3 million hectares out of the total of 6.8 million salt-affected agricultural lands have been only affected by salinity, and face no other environmental limitations for sustainable crop production (Moameni, 2011). Today, mainly due to human activity, soil salinization is increasing. One big change salt causes is a disturbance of osmotic water balance, thereby increasing the concentrations

* Corresponding author. Tel.: +98 912 2536674
Fax: +98 25 32126488
E-mail address: tavakolineko@ut.ac.ir

of toxic ions which result in membrane disorganization, ion toxicity, and oxidative stress (Hasegawa *et al.*, 2000; Ottow *et al.*, 2005; Zhu, 2001). *Populus euphratica* is a tree with significant tolerance against salt, drought, and extreme temperature stresses (Wang *et al.*, 2008). This tree can survive for tens of years in harsh saline and arid environments, and can grow under extreme conditions such as saline and alkaline soils (Chen *et al.*, 2002; Kang *et al.*, 1996; Ottow *et al.*, 2005; Watanabe *et al.*, 2001). It is native to the semi-arid areas ranging from northwestern China to western Morocco (Browicz, 1977). . *P. euphratica*, like other species of *Populus*, subtends ecological effects such as improved microclimatic conditions, erosion control, shade or shelter, reclamation, phytoremediation, ornamental and intercropping. Its wood is used for house construction, timber, fuel wood and fiber and its leaves are used for fodder; thus, it lays an important role in the local economy. It so follows that this tree is very useful for afforestation on saline and alkaline desert areas. *P. euphratica* is important for stabilizing sand dunes and constructing agricultural greenbelts (Wei, 1993). In addition to its economic and ecological significance, *Populus* is used as a model in physiological and molecular studies on stress tolerance in tree species (Chen and Polle, 2010). *P. euphratica* can withstand up to to 450 mM NaCl under hydroponic conditions and can tolerate high Na^+ and Cl^- concentrations in its roots and leavesafter exposure to a salinity of 300 mM NaCl for 1 month (Chen and Polle, 2010; Gu *et al.*, 2004). Plants possess many defense responses to cope with salinity at different levels; at the whole plant level for example, one strategy sees many non-halophytes exclude salt (Greenway and Munns, 1980); however, this strategy is not important in *P. euphratica*, which showed no restriction of Na^+ uptake into its roots (Chen *et al.*, 2001). At the cellular level, the metabolic response to salt stress is the synthesis of antioxidant enzymes, chaperones and compatible solutes (Hasegawa *et al.*, 2000; Wang *et al.*, 2008). The ability of *P. euphratica* to produce osmolytes seems to be limited (Brosche *et al.*, 2005). There is proof that Na^+ acts as a cheap and available osmolyte in *P. euphratica* (Ottow *et al.*, 2005). Poplar roots have been shown to accumulate higher Na^+ concentrations than the leaves. Therefore, the roots have the ability to retain considerable amounts of Na^+. It has been claimed that Cl^- toxicity is more damaging than Na^+ toxicity in some woody species, e.g., Citrus (Manns and Tester, 2008). In genetic variations of *P. euphratica* a fascinating question is how trees, which have a long life span and, thus, must be able to cope with abundant salt for longer periods of time, adapt to high salinity. *P. euphratica* is a model for salt tolerance in trees (Chen and Polle, 2010). The natural habitats of *P. euphratica* covered a vast area of Iran in last decade (Rechinger, 1969), but because of some threats and anthropogenic damage, its coverage, currently, is limited to some parts of west, north-west, south-west, south east, and central parts of Iran as scattered stands (Mohammadi *et al.*, 2013). Its wide distribution in the world and in Iran with a vast range of climate variability and, especially, soil and salinity, is probably due to its potentially genetic variations in its resistance to salinity. The objectives of this research are the evaluation and comparison of different *P. euphratica* ecotypes in Iran in terms of salt tolerance, how the salts accumulate in different organs of this plant and where it exhibits resistance to salinity.

2. Materials and Methods

2.1. Study area

In mid-February, 1-year-old cuttings of *P. euphratica* were collected from 12 regions of Iran. Table 1 shows the locations and properties of the collection areas and Fig.1 illustrates the collection areas on a map of Iran.

2.2. Sampling

The cuttings were planted in individual pots containing Sandy-loam soil in a nursery at the University of Tehran and placed in a greenhouse; the cuttings rooted in April. The plants were irrigated 2-3 times per week, depending on evaporative demand and received 1 liter of full-strength Hoagland's nutrient solution every 2 weeks. Rooted cuttings were maintained in the greenhouse for hardening and acclimation for 6 months prior to the initiation of salt treatments (October). 180 uniform plants in height and number of leaves were used in the following experiment.

2.3. Stress treatments

Plants were subjected to increasing salinity for 2 months, and the saline treatments were imposed by top watering with 1 liter of 75, 150, 225, and 300 mM NaCl solutions twice a week. When salt treatments were initiated, plants received 1 L of full-strength Hoagland's solution on a weekly basis. Control plants were kept

well-watered with distilled water and fertilized with no addition of NaCl. Destructive harvests were made after 2 months of exposure to salt treatments. Three replicated seedlings per treatment were harvested at each sampling time.

Table 1. Characteristics of ecotypes studied

Name of region	Province	Symbol	Longitude	Latitude	Elevation (m)	Mean annual temperature (°C)	Mean annual precipitation (mm)
Jolfa	Azerbaijan	E1	38 57 N	45 41 E	0703	14.4	179.8
Marand	Azerbaijan	E2	38 31 N	45 24 E	1077	12.3	342.2
Maranjab	Esfahan	E3	34 13 N	51 40 E	0930	18.8	138.4
Manjil	Gilan	E4	36 15 N	49 26 E	0330	17.3	196.4
Dashlibrun	Golestan	E5	37 46 N	54 54 E	0037	17.1	201.9
Sarakhs	Khorasan	E6	36 18 N	61 09 E	0303	17.6	203.3
Dezful	Khuzestan	E7	32 14 N	48 20 E	0063	24.0	444.3
Hamidieh	Khuzestan	E8	31 31 N	48 28 E	0023	24.2	194.5
Mahalat	Markazi	E9	34 00 N	50 33 E	1850	12.8	294.2
Masumieh	Qom	E10	34 43 N	50 52 E	0910	18.7	146.1
Gilvan	Zanjan	E11	36 46 N	49 26 E	0376	17.3	196.4
Mahneshan	Zanjan	E12	36 46 N	47 43 E	1706	14.6	207.0

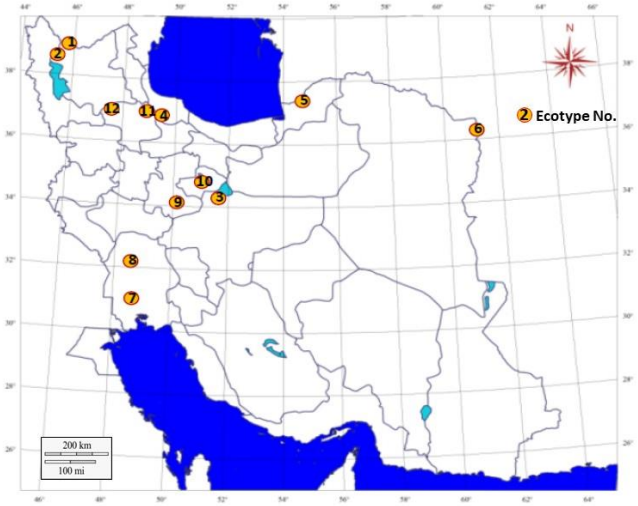

Fig. 1. Location of ecotypes studied

2.4. Traits Assessment

Growth measurements were started 1 day before the salt treatments. Freshness class, collar diameter, shoot height, and biomass production were measured before and after salt treatments. To convert qualitative to quantitative data of freshness, the seedlings were grouped in terms of freshness into 4 classes (4=complete freshness and 0=completely withered). Height growth was measured from the growth tip to the base of the stem. Collar diameter was measured at the base of the stem. Diameter (Di) and height (Hi) growth rates were calculated. Leaf, stem, and root dry weights were weighed to 0.001 g after 48 hours in an oven at 75°C.

At each harvest time, soil samples were taken at 20–30 cm depth from the pots and soil water content was determined for all treatments and the control pots. Saturated paste extracts were used for EC_e and pH measurements. Extracts of the soil samples (dried soil: Sodium-polyphosphate 1N = 1:1, w/v) were used for Na^+, K^+, Ca^{2+} and Mg^{2+} measurements. Extracts of the soil samples (dried soil: deionized water = 1:1, w/v) were used for Cl^- determination. Extracts of the soil samples (dried soil: Sodium-bicarbonate 0.5 M (pH=8) = 1:2, w/v) were used for available Phosphorus (P) as per the Olsen method (Olsen *et al*., 1954). Na^+ and K^+ were measured by flame photometer (Jeenmay PFP7; UK), Ca^{2+} and Mg^{2+} were measured by complexometric titration, P was measured by spectrophotometer at 630 nm (Shimatsu UV-160A; Japan) and Cl^- was measured by silver titration ($AgNO_3$ solution). The pH and EC_e were measured using extracts of the saturated soil.

A destructive harvest was done after 60 days of exposure to the initial salt treatments. Three cuttings per treatment were harvested. Fully

expanded leaves were sampled from upper portions of the shoots. The roots were thoroughly washed free of soil with deionized water and fine roots (diameter <1 mm) were sampled for mineral analysis. All sampled materials (roots and leaves) were oven-dried (48 hours at 75°C) until a dry biomass was obtained. Dried samples were ground into a powder and stored for mineral analysis**.**

Duplicated 1 g samples were at 600 °C for 6 hours and digested, and extracts of the plant samples (HCl 2*N* : H_2O = 1:10, v/v) were used to determine Na^+ and K^+ concentrations by flame photometer (Jeenmay PFP7; UK) and Ca^{2+} and Mg^{2+} by complexometric titration .

Extracts of plant tissue samples (dried plant: deionized water = 1:100, w/v) were used to determine Cl^- concentration by way of a modified method of silver titration ($AgNO_3$ solution).

Duplicated 1 g samples were ashed at 500 °C for 6 hours and digested, and extracts of the plant samples (HCl 2*N* : H_2O = 1:10, v/v) were used for the colorimetric determination of *P* as vanadate-ammonium molybdate reagent (vanadate-yellow color method) by spectrophotometer (Shimatsu UV-160A; Japan) at 450 nm (Cavell, 1955).

2.5. Statistical analysis

Statistical analysis was performed using the Statistical Analysis System (9.1 SAS Institute Inc.). One-factor ANOVA was performed to identify statistically significant differences between different sampling levels of salinity and ecotypes; significant differences between the means were determined by Duncan's multiple-range test.

3. Results

The NaCl concentration in the soil subjected to salt (1 L of 75 to 300 mM NaCl) changed some parameters such as EC_e and some ions, but not the pH. Also the NaCl concentration in the soil subjected to salt (1 L of 75 to 300 mM NaCl) changed some parameters such as ion concentration and SAR (Table 2).

Table 2. Ion concentration of salinized soil and control (non-salt)

Treatments	EC dS/m	pH	Na^+ mmol /kg	K^+ mmol /kg	Ca^{2+} mmol /kg	Mg^{2+} mmol /kg	N mmol /kg	P mmol /kg	$CO3^{2-}$ mmol/kg	HCO^{3-} mmol/kg	Cl^- mmol /kg	SAR
Control	0.15	7.1	5.88	0.35	0.50	0.30	21.43	1.86	0	1.88	0.80	5.77
1	2.17	7.6	25.20	0.32	0.80	0.40	28.57	2.12	0	2.01	3.40	23.01
2	3.81	7.5	35.35	0.30	1.20	0.80	50.00	3.82	0	2.10	5.11	26.64
3	5.23	7.5	39.99	0.35	1.40	1.00	58.57	3.45	0	2.15	7.01	28.28
4	6.16	7.8	79.99	0.40	1.60	0.80	64.29	4.44	0	2.67	11.62	53.93

Each value is the mean of three replications.

Variance analysis showed that salinity levels were significantly different (P≤0.001) in treatments and ecotypes, but for teats × ecotypes interaction, it varied in each variable. There were significant differences (P≤0.001) in performance indices [diameter and height growth, biomass production; leaf, stem and root biomass (dry weight) and total biomass] at different salinity levels and different ecotypes. In general, the coefficients of variation for these variables ranged between 2.12% to 9.08%, which indicate good to fair results depending on the type of experiments and measuring methods used. Variance analysis showed that concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^- and *P* were significantly different (P≤0.001) due to the salinity levels in the treatments and did not have a significant difference (P≤0.05) on salinity × ecotypes interaction; but these variables varied for different ecotypes and there were no significant differences in most of them. Generally, the coefficients of variation for these variables ranged from 2.27% to 29.17%.

Duncan's Multiple Range Test for variables in different ecotypes showed that ecotype (5) was in the highest group in terms of freshness and ecotype (8) was in the highest group in growth and productivity indices; in contrast, ecotype (2) was in the lowest group in freshness and most of the growth and productivity indices; also ecotype (12) was in the lowest group with regards to root dry weight.

The same test indicated that all 12 ecotypes were classified in a few groups. Concerning macronutrients and ions in leaves and roots: ecotypes 4 and 8 were in the highest group with regards to macronutrients (K^+, *P*),; although Ca^{2+} and Mg^{2+} were not significantly different (P≤0.05); the highest salt ions ($Na+$, Cl^-) were found in ecotype (2); ecotypes 2 and 12 had the lowest amount of nutrient elements such as K^+ and *P*. In contrast, the lowest groups of Na^+ were in ecotypes 6 and 4, although the Cl^- did not have significant variance (P≤0.05)(Table 3). Duncan's Multiple Range Test for variables in different salt levels showed that the highest

freshness and diameter growth were those of the control specimens, whereas, the cuttings belonging to 75 mM NaCl treatment were in the highest group regarding performance traits such as height growth, biomass production; leaf, stem, root and total biomass (dry weight). In contrast, the lowest freshness and growth traits were observed for cuttings undergoing 300 mM NaCl treatment. Overall, the highest total biomass (dry weight) was observed for cuttings of the 75 mM NaCl treatment group with 37.75 g and the lowest total biomass (dry weight) was observed for cuttings treated with 300 mM NaCl with 30.08 g. Duncan's Multiple Range Test for variables in different treatments of salt levels indicated that all of the 12 ecotypes were classified into 5 groups from those with the highest ion concentration, except those of Ca^{2+} and Mg^{2+} in leaves and roots. Ca^{2+} and $Mg2^{+}$ did not have a sharp trend in different treatments of salt levels, and the cuttings were separated into fewer groups. The highest concentrations of nutrient elements such as K^{+} and *P* in the leaf and root were observed in the control specimens, followed by those treated with75 mM NaCl; in contrast the highest concentrations of Na^{+} and Cl^{-} in leaves and roots were in the 300 mM NaCl treatment, wgucg aksi had the lowest concentration of nutrient elements such as K^{+} and *P*; in contrast, the control cuttings had the lowest concentrations of Na^{+} and Cl^{-} in the leaves and roots (Table 4).

Pearson Correlation Coefficients analysis showed a strong and positive correlation (P≤0.001) between performance indices [diameter and height growth, biomass production; leaf, stem and root biomass (dry weight) and total biomass]. In leaves and roots, there was a strong and positive correlation (P≤0.001) between performance indices with K^{+} and *P*, a strong and negative correlation (P≤0.001) between performance indices with Na^{+} and $Cl^{-}$and no any correlation (P≤0.05) between performance indices with Ca^{2+} and Mg^{2+}. Although, there was a strong and positive correlation (P≤0.001) between macronutrients (K^{+} and *P*).

There was a strong and positive correlation (P≤0.001) between salt ions (Na^{+} and Cl^{-}). Whereas, there was a strong and negative correlation (P≤0.001) between macronutrients (K^{+} and *P*) with salt ions (Na^{+} and Cl^{-}). Also, there was a strong and positive correlation (P≤0.001) between Ca^{2+} with Mg^{2+} in both leaves and roots. There was no any correlation (P≤0.05) between K^{+} and *P* with Ca^{2+} and Mg^{2+} (Table 5).

4. Discussion

Salt increase (Control to 300mM NaCl) caused the increase in EC of soil from 0.63 to 6.70 dS/m, but did not affect soil pH, which varied between 7.02 and 7.45. The salt ions (Na^{+}, Cl^{-}) were strongly enhanced by increasing salt levels with a high concentration of these ions in irrigation water, which resulted in their high concentration in the soil. Also, the macronutrients (K^{+}, Ca^{2+}, Mg^{2+}, *N* and *P*) were enhanced with an increase in salt levels, due to the disturbance of ion absorption by plants under conditions of high salinity.

P. euphratica is greatly adapted to hostile desert environments and is an important model species in the assessment of physiological and molecular effects of abiotic stresses (Chen *et al.*, 2001). The effects of salt levels and ecotypes on performance indices showed significant difference, while no significant difference was seen in salt × ecotype. The effects of salt levels on ion concentrations showed significant difference (except for Ca^{2+}); the ecotype effects were varied and some ions showed significant differences while others were not different. Whereas the salt × ecotype interaction of ion concentrations had no significant difference, it showed that the interaction of the two factors was independent. The results of this experiment indicated that *P. euphratica* is a salt-tolerant Poplar species despite being a non-halophyte. This agrees with the conclusions of (Wang *et al.*, 2008), but *P. euphratica* calluses show some similarity to halophytes in terms of salt adaptation (Zhang *et al.*, 2007). In the present study, the maximum growth rate was observed at 75 mM NaCl, which agrees with *Zhang et al.*, (2007) who reported the maximum growth rate at 50 mmol/l NaCl.

Table 3. Duncan's Multiple Range Test for variables in ecotypes

Variable	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
Freshness (class)	3.7100^{bc}	3.6700^{c}	3.7300^{bac}	3.7100^{bc}	3.7800^{a}	3.7100^{bc}	3.6900^{bc}	3.7500^{ba}	3.7100^{bc}	3.7400^{ba}	3.7100^{bc}	3.6800^{bc}
Diameter growth (mm)	0.3300^{ba}	0.2300^{d}	0.3300^{ba}	0.3400^{ba}	0.3300^{ba}	0.3300^{ba}	0.3100^{bc}	0.3400^{a}	0.3200^{bc}	0.3400^{a}	0.3300^{ba}	0.3000^{c}
High growth (cm)	21.6000^{a}	18.7600^{d}	24.3100^{b}	25.790^{a}	21.8200^{c}	21.9700^{c}	22.0400^{c}	26.1500^{a}	22.1400^{c}	26.0100^{a}	22.1200^{c}	19.4100^{d}
Biomass production (g)	8.1300^{cb}	6.7900^{d}	10.3300^{a}	10.3000^{a}	8.2000^{cb}	8.5400^{b}	8.1400^{cb}	10.9000^{a}	8.4400^{b}	10.5400^{a}	8.4400^{b}	7.6200^{c}
Leaf dry weight (g)	13.6000^{bc}	11.8600^{f}	13.0600^{c}	13.9800^{ba}	13.0900^{d}	13.8300^{ba}	13.2000^{dc}	14.1800^{a}	13.3600^{dc}	13.8800^{ba}	12.5600^{e}	12.6100^{e}
Stem dry weight (g)	12.9500^{dc}	11.7500^{f}	13.0100^{bdc}	12.9700^{dc}	12.6900^{edc}	13.1000^{bac}	12.5400^{e}	13.4500^{a}	12.7100^{edc}	13.3800^{ba}	12.6600^{ed}	11.8700^{f}
Root dry weight (g)	8.7400^{a}	8.1500^{ab}	8.9300^{a}	8.8900^{a}	8.7800^{a}	8.8900^{a}	8.7600^{a}	9.0000^{a}	8.7800^{a}	8.9400^{a}	8.0400^{b}	8.0100^{b}
Total dry weight (g)	35.2900^{bdc}	31.7600^{f}	35.0100^{dc}	35.8400^{bac}	34.5600^{d}	35.8200^{bac}	34.5000^{d}	36.6400^{a}	34.8600^{dc}	36.2000^{ba}	33.2600^{e}	32.4800^{fe}
Na^{+} ($mmol.g^{-1}_{DW\ Leaves}$)	0.4350^{bc}	0.4590^{a}	0.4320^{ba}	0.4350^{ba}	0.4340^{ba}	0.4290^{c}	0.4480^{bac}	0.4370^{bc}	0.4470^{bac}	0.4350^{bc}	0.4390^{bac}	0.4530^{ba}
K^{+} ($mmol.g^{-1}_{DW\ Leaves}$)	1.1920^{a}	1.1570^{b}	1.2000^{a}	1.2050^{a}	1.1890^{a}	1.1970^{a}	1.1880^{a}	1.2070^{a}	1.1950^{a}	1.1990^{a}	1.1950^{a}	1.6000^{b}
Ca^{2+} ($mmol.g^{-1}_{DW\ Leaves}$)	0.2750^{a}	0.2710^{a}	0.2760^{a}	0.2730^{a}	0.2740^{a}	0.2750^{a}	0.2710^{a}	0.2750^{a}	0.2740^{a}	0.2760^{a}	0.2730^{a}	0.2690^{b}
Mg^{2+} ($mmol.g^{-1}_{DW\ Leaves}$)	0.1750^{a}	0.1710^{b}	0.1740^{a}	0.1750^{a}	0.1740^{a}	0.1750^{a}	0.1750^{a}	0.1750^{a}	0.1740^{a}	0.1740^{a}	0.1730^{a}	0.1710^{a}
Cl^{-} ($mmol.g^{-1}_{DW\ Leaves}$)	0.0470^{a}	0.0490^{a}	0.0450^{a}	0.0440^{a}	0.0470^{a}	0.0450^{a}	0.0470^{a}	0.0450^{a}	0.0470^{a}	0.0440^{a}	0.0460^{a}	0.0470^{a}
P ($\mu mol.g^{-1}_{DW\ Leaves}$)	0.0271^{bac}	0.0261^{d}	0.0267^{bdac}	0.0273^{ba}	0.0267^{bdac}	0.0271^{ba}	0.0264^{bdc}	0.0274^{a}	0.0271^{bac}	0.0273^{ba}	0.0262^{dc}	0.0259^{d}
Na^{+} ($mmol.g^{-1}_{DW\ Roots}$)	0.2600^{ba}	0.2700^{a}	0.2500^{ba}	0.2500^{b}	0.2600^{ba}	0.2600^{ba}	0.2600^{ba}	0.2500^{ba}	0.2500^{ba}	0.2500^{ba}	0.2600^{ba}	0.2600^{ba}
K^{+} ($mmol.g^{-1}_{DW\ Roots}$)	0.4700^{ba}	0.4600^{bc}	0.4700^{ba}	0.4800^{a}	0.4700^{ba}	0.4700^{ba}	0.4600^{bc}	0.4800^{a}	0.4700^{ba}	0.4800^{ba}	0.4700^{ba}	0.4500^{c}
Ca^{2+} ($mmol.g^{-1}_{DW\ Roots}$)	0.1800^{a}	0.1790^{a}	0.1810^{a}	0.1810^{a}	0.1800^{a}	0.1800^{a}	0.1800^{a}	0.1820^{a}	0.1800^{a}	0.1810^{a}	0.1800^{a}	0.1790^{a}
Mg^{2+} ($mmol.g^{-1}_{DW\ Roots}$)	0.8900^{a}	0.8500^{a}	0.8900^{a}	0.8900^{a}	0.8900^{a}	0.8900^{a}	0.8700^{a}	0.9100^{a}	0.8900^{a}	0.9100^{a}	0.8700^{a}	0.8100^{a}
Cl^{-} ($mmol.g^{-1}_{DW\ Roots}$)	0.0250^{b}	0.0280^{a}	0.0260^{ba}	0.0260^{ba}	0.0250^{b}	0.0270^{ba}	0.0270^{ba}	0.0250^{b}	0.0270^{ba}	0.0260^{b}	0.0270^{ba}	0.0270^{ba}
P ($\mu mol.g^{-1}_{DW\ Roots}$)	0.0096^{ebdac}	0.0081^{f}	0.0101^{bac}	0.0097^{bdac}	0.0095^{edc}	0.0097^{bdac}	0.0091^{ed}	0.0104^{a}	0.0095^{ebdc}	0.0103^{ba}	0.0097^{bdac}	0.0088^{ef}

Means with the same letter in the rows are not significantly different at $P \leq 0.05$

Table 4. Duncan's Multiple Range Test for variables in treatments of salt levels

Variable	Treatments				
	Control	75 mM	150 mM	225 mM	300 mM
Freshness (class)	3.970^{a}	3.960^{a}	3.900^{b}	3.890^{b}	2.870^{c}
Diameter growth (mm)	0.350^{a}	0.340^{ba}	0.330^{bc}	0.320^{c}	0.270^{d}
High growth (cm)	25.470^{b}	26.330^{a}	24.040^{c}	21.610^{d}	15.930^{e}
Biomass production (g)	9.830^{a}	9.890^{a}	9.330^{b}	8.740^{c}	6.530^{d}
Leaf dry weight (g)	13.530^{b}	14.470^{a}	13.390^{b}	13.020^{c}	11.920^{d}
Stem dry weight (g)	13.670^{b}	14.200^{a}	13.540^{b}	11.990^{c}	10.380^{d}
Root dry weight (g)	8.930^{ba}	9.080^{a}	8.910^{ba}	8.600^{b}	7.780^{c}
Total dry weight (g)	36.130^{b}	37.750^{a}	35.840^{b}	33.620^{c}	30.080^{d}
Na^{+} ($mmol.g^{-1}_{DW\ Leaves}$)	0.054^{e}	0.459^{d}	0.496^{c}	0.538^{b}	0.645^{a}
K^{+} ($mmol.g^{-1}_{DW\ Leaves}$)	1.347^{a}	1.234^{b}	1.206^{c}	1.144^{d}	1.021^{e}
Ca^{2+} ($mmol.g^{-1}_{DW\ Leaves}$)	0.271^{b}	0.269^{c}	0.273^{cb}	0.280^{a}	0.273^{cb}
Mg^{2+} ($mmol.g^{-1}_{DW\ Leaves}$)	0.176^{a}	0.173^{a}	0.174^{a}	0.173^{a}	0.173^{a}
Cl^{-} ($mmol.g^{-1}_{DW\ Leaves}$)	0.020^{d}	0.041^{c}	0.044^{c}	0.055^{b}	0.071^{a}
P ($\mu mol.g^{-1}_{DW\ Leaves}$)	0.0328^{a}	0.0317^{b}	0.0274^{c}	0.0246^{d}	0.0174^{e}
Na^{+} ($mmol.g^{-1}_{DW\ Roots}$)	0.056^{e}	0.193^{d}	0.255^{c}	0.333^{b}	0.446^{a}
K^{+} ($mmol.g^{-1}_{DW\ Roots}$)	0.501^{b}	0.511^{a}	0.478^{c}	0.461^{d}	0.399^{e}
Ca^{2+} ($mmol.g^{-1}_{DW\ Roots}$)	0.158^{c}	0.166^{cb}	0.177^{cb}	0.216^{a}	0.183^{b}
Mg^{2+} ($mmol.g^{-1}_{DW\ Roots}$)	0.079^{c}	0.082^{c}	0.085^{bc}	0.096^{ba}	0.099^{a}
Cl^{-} ($mmol.g^{-1}_{DW\ Roots}$)	0.009^{e}	0.020^{d}	0.025^{c}	0.035^{b}	0.043^{a}
P ($\mu mol.g^{-1}_{DW\ Roots}$)	0.013^{a}	0.012^{b}	0.010^{c}	0.008^{d}	0.006^{e}

Means with the same letter in the rows are not significantly different at $P \leq 0.05$.

Table 5. Pearson Correlation Coefficients analysis between variables

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
C1	1.00																			
C2	0.68	1.00																		
C3	0.76	0.81	1.00																	
C4	0.65	0.81	0.91	1.00																
C5	0.61	0.77	0.86	0.79	1.00															
C6	0.79	0.80	0.90	0.81	0.84	1.00														
C7	0.51	0.53	0.59	0.55	0.58	0.62	1.00													
C8	0.74	0.81	0.90	0.82	0.92	0.95	0.78	1.00												
C9	-0.56	-0.54	-0.54	-0.46	-0.35	-0.57	-0.33	-0.49	1.00											
C10	0.80	0.74	0.79	0.69	0.62	0.84	0.51	0.76	-0.90	1.00										
C11	0.03	0.24	0.09	0.16	0.12	-0.03	0.11	0.06	0.11	-0.06	1.00									
C12	0.09	0.43	0.23	0.33	0.33	0.24	0.26	0.31	-0.13	0.19	0.47	1.00								
C13	-0.73	-0.70	-0.72	-0.64	-0.56	-0.78	-0.47	-0.71	0.90	-0.96	0.09	-0.27	1.00							
C14	0.86	0.72	0.83	0.69	0.71	0.89	0.54	0.83	-0.78	0.95	-0.12	0.16	-0.90	1.00						
C15	-0.75	-0.64	-0.73	-0.60	-0.55	-0.78	-0.44	-0.69	0.92	-0.97	0.17	-0.11	0.95	-0.94	1.00					
C16	0.84	0.79	0.87	0.77	0.78	0.91	0.58	0.88	-0.62	0.86	0.06	0.34	-0.81	0.93	-0.81	1.00				
C17	0.01	0.30	-0.03	0.11	0.11	-0.01	0.05	0.05	0.19	-0.10	0.52	0.43	0.10	-0.12	0.22	0.04	1.00			
C18	-0.20	0.20	-0.09	0.05	0.07	-0.06	0.01	0.00	0.20	-0.16	0.54	0.42	0.14	-0.22	0.26	-0.06	0.86	1.00		
C19	-0.73	-0.65	-0.74	-0.61	-0.57	-0.80	-0.47	-0.72	0.89	-0.96	0.17	-0.16	0.95	-0.94	0.98	-0.83	0.22	0.26	1.00	
C20	0.76	0.77	0.83	0.73	0.71	0.87	0.54	0.82	-0.77	0.91	-0.07	0.22	-0.87	0.93	-0.89	0.89	-0.08	-0.15	-0.91	1.00

The correlation coefficient above 0.25, 0.20, and 0.15 are significantly different at $p<0.001$, $p<0.01$, $p<0.05$, respectively, and below 0.15 are not significantly different at $P\leq0.05$.

C1: Freshness (class); C2: Diameter growth (mm); C3: High growth (cm); C4: Biomass production (g); C5: Leaf dry weight (g); C6: Stem dry weight (g); C7: Root dry weight (g); C8: Total dry weight (g); C9: Na^{+} ($mmol.g^{-1}_{DW\ Leaves}$); C10: K^{+} ($mmol.g^{-1}_{DW\ Leaves}$); C11: Ca^{2+} ($mmol.g^{-1}_{DW\ Leaves}$); C12: Mg^{2+} ($mmol.g^{-1}_{DW\ Leaves}$); C13: Cl^{-} ($mmol.g^{-1}_{DW\ Leaves}$); C14: P ($\mu mol.g^{-1}_{DW\ Leaves}$); C15: Na^{+} ($mmol.g^{-1}_{DW\ Roots}$); C16: K^{+} ($mmol.g^{-1}_{DW\ Roots}$); C17: Ca^{2+} ($mmol.g^{-1}_{DW\ Roots}$); C18: Mg^{2+} ($mmol.g^{-1}_{DW\ Roots}$); C19: Cl^{-} ($mmol.g^{-1}_{DW\ Roots}$); C20: P ($\mu mol.g^{-1}_{DW\ Roots}$)

The salt ions (Na^+, Cl^-), were enhanced with simultaneous salt treatments in plant leaves and roots; but the accumulation of these ions in plant tissues was less than that in the soil; this indicates that there are some mechanisms to exclude salt or strategies for its excretion; previous research is compatible with these recent results (Zeng *et al.*, 2009). The *P. euphratica's* potential to tolerate high salt concentrations is because of its high capacity to exclude Na^+ and Cl^- ions at the root level (Sun *et al.*, 2010). *P. euphratica* can tolerate a great deal of salt in its leaves (Arndt *et al.*, 2004), and this has been attributed to the compartmentalization of Na^+ into the apoplast (Ottow *et al.*, 2005). The macronutrients (K^+, *P*) decreased with simultaneous salt treatment in the plant leaves and roots; but the accumulation of these ions in plant tissue was much more than that in the soil; this showed that *P. euphratica* had a comparatively higher amount of net uptake and transport of K^+. Thus, *P. euphratica* retained a high capacity for K^+ uptake and transport in the presence of high external Na^+ concentrations, which appeared to result from preferential uptake of K^+ vis-à-vis Na^+ (Flowers *et al.*, 1977; Mills *et al.*, 1985). The macronutrients (Ca^{2+}, Mg^{2+}) were enhanced with simultaneous salt treatments in the leaves and roots, except for leaf Mg^{2+} which was not significantly different ($P \leq 0.05$), this was in agreement with Chen *et al.*, (2001). The Na^+ current in the maize root's plasma membrane was to some extent restrained by extracellular Ca^{2+} (Roberts and Tester, 1997). Ca^{2+} inhibited Na^+ influx more effectively in a hexaploid variety of wheat, which is more salt-tolerant than tetraploid varieties (Allen *et al.*, 1995).

There was a strong and positive correlation between performance indices and macronutrients, especially K^+ and *P*. On the other hand, there was a strong and positive correlation between macronutrients, especially K^+ and *P*; this proved that there is synergism taking place during ion uptake. There was a strong and negative correlation between macronutrients (especially, K^+ and *P*) and salt ions (Na^+ and Cl^-); this proved that there was some competition in the uptake of these ions. The decreased K^+ is the result of a competitive process between K^+ and Na^+ (Grattan and Grieve, 1992; Subbarao *et al.*, 1990). Also, there was a strong and positive correlation between the salt ions Na^+ and *Cl*); this proved the existence of synergy in the uptake of these ions. There was a weak and positive correlation between macronutrient Ca^{2+} and salt ions Na^+,which showed an adaptive mechanism in this species for salt tolerance. It is probable that the high level of Ca^{2+} in the root of *P. euphratica* could contribute to retain a high capacity of limiting salt uptake and transport under salt stress, because Ca^{2+} is crucial for preserving the structural integrity and selective permeability of root membranes (Cramer *et al.*, 1985).

5. Conclusions

Although *P. eupratica* is not a well-known halophyte, it displays some characteristics of halophytes, of which we can point out the maximum growth rate of *P. eupratica* in low salt conditions compared with the control (non-salt). This plant has multiple mechanisms to deal with salt stress. The key mechanisms of salt tolerance in this plant are as follows: firstly, excluding Na^+ and Cl^- from the roots. Secondly, compartmentalization of Na^+ and Cl^- in the roots and leaves under stressful conditions. Thirdly, stopping the excessive reduction of macronutrient K+.

There is evidence that in *P. eupratica*, Na^+ acts like an osmolyte. The role of Na^+ as an osmolyte is cheaper than compatible solutes, as their synthesis is more expensive energy wise . When the plant is subjected to excessive salt, it needs to reduce osmotic potential for uptake water. As the research shows, the accumulation of salt in the tissues of the plant and ability to compartmentalize ions can be present as mechanism for salt tolerance both in halophytes and glycophytes. *P. eupratica* is able to absorb K^+ ions in the presence of high concentrations of Na^+ and transport it to the tissues. The prevention of excessive reduction of K^+ will enhance the K^+ / Na^+ balance.

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