Plant Omics Journal POJ

POJ 5(3):271-278 (2012) ISSN:1836-3644

Salinity effects on macro and micronutrients uptake in medicinal plant King of Bitters (*Andrographis paniculata* Nees.)

Daryush Talei[1, 2,*], Mihdzar Abdul Kadir[3], Mohd Khanif Yusop[4], Alireza Valdiani[3] and Mohd Puad Abdullah[1]

[1]Department of Cell and Molecular Biology, Faculty of Biotechnology & Biomolecular, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[2]Medicinal Plant Research Centre, Shahed University, Tehran, Iran
[3]Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[4]Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

***Corresponding author: khanif@agri.upm.edu.my and Talei1348@gmail.com**

Abstract

Plants species require macro and micro nutrients to complete the growth cycles such as photosynthesis, enzyme activities and secondary metabolite production. The objective of the present study was to investigate the effects of salinity on biomass as well as macro and micro nutrients uptake in the medicinal plant of *Andrographis paniculata* (AP). In this regard, a split plot experiment was carried out based on Randomized Complete Block Design (RCBD) with two factors; five salinity levels (control, 4, 8, 12 and 16 dsm^{-1}) in main plots and 12 different AP accessions in sub-main plots with three replicates. Seventy-day-plants (before flowering stage) were exposed to different salinity levels on a Hoagland medium. The results indicated that salinity levels had a significant effect on the measured traits. Salinity caused a reduction in dry material and salt tolerance index (STI). The sodium (Na^+), iron (Fe), zinc (Zn) and copper (Cu) contents significantly increased, while nitrogen (N), phosphorus (P), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) and manganese (Mn) content decreased in high salinity levels. Furthermore, under salinity stress, tolerant accessions could produce higher K^+, P, N, Mg^{2+} and Ca^{2+} and lower Na^+, Fe, Zn and Cu than sensitive accessions. Interestingly, Na^+ concentration was highly correlated to STI ($r = -0.79$), while the lowest correlation was observed between P and Na^+ concentration ($r = -0.15$). Salt-stress caused changes in macro and micro nutrients uptake, which may lead to decline in photosynthesis capacity and respiration. This type of abiotic stress can also disturb metabolism of several cellular components such as protein synthesis.

Keywords: *Andrographis paniculata*, salinity stress, salt tolerance, macro and micronutrients.
Abbreviations: CHLO, chlorophyll; NL, number of leave; RL, root length; SL, shoot length; STI, salt tolerance index; TDW, total dry weight.

Introduction

Soil salinity is a major environmental factor causes reduction in plant growth and productivity in arid and semiarid areas of the world. Approximately, 20% of the world's cultivated area and about half of the world's irrigated lands are reported to be seriously affected by salinity and water logging (Munns and Tester, 2008; FAO, 2008). In general, high salt concentration in soils causes reduction in osmotic potential, which lead to disturb water availability to root cells and make difficulties for plant to obtain both water as well as nutrients. Consequently, a rapid reduction happens in growth rate, productivity and many metabolic changes due to hormonal signal generated by the roots (Munns, 2002; Ashraf and Harris, 2004).

Researchers have indicated that plant species have different abilities to tolerate salinity stress, which depends on several interacting variables such as the plant growth stage, salt concentration and the duration of stress over time (Munns, 2002; Vallejo et al., 2010; Zhu, 2002). The deleterious effects of salinity on plant growth is attributed to the decrease in osmotic potential of the growing medium, specific ion toxicity and nutrient ion deficiency by disrupting potassium (K^+) nutrition, which can be a result of inorganic ion (Na^+, Cl^-, and K^+) and compatible organic solute accumulations (soluble carbohydrates, amino acids, proline, betaines, etc.) (Luo et al., 2005; Hasegawa et al., 2000). The osmotic adjustment in both roots and leaves contribute to the maintenance of water uptake and cell turgor, allowing physiological processes, such as stomata opening, photosynthesis, and cell expansion (Serraj and Sinclair, 2002). It is reported that the root growth and total fresh weight of root were more adversely affected by salinity compared to shoot growth (Parida and Das, 2005). Eventually, responses of plant roots and shoots to soil salinity should be understood both under salinity conditions. Besides, there is the possibility that many nutrient interactions in salt-stressed plants may have important consequences to growth.
Plants require macro and micro elements to grow. In soil, micronutrients are not always present in the solution and their

availability is limited due to several factors, which mainly limit their solubility. The main factors determining the level of soluble Macro- and micronutrients in soil solution are: Oxidation-reduction reactions and the pH. Some of the interactions interfere and damage the availability of micronutrients. Some of the interactions are soil interactions, and some others happen in the plant tissue due to excessive concentrations. Internal concentrations of major nutrients and their uptake have been frequently studied (Maas and Grieve, 1987; Cramer et al., 1987), but the relationship between macro and micronutrient concentration under salinity condition is rather complex and remains poorly understood (Tozlu et al., 2000). *Andrographis paniculata* belongs to family Acanthacea is an important medicinal plant (Valdiani et al., 2012). The plant exhibited a spacious capacity of pharmaceutical properties such as anti-HIV (Basak et al., 2006), anti-H1N1 (Ko et al., 2006), anticancer (Chun et al., 2010) and anti-hepatitis (Dumrongsak et al., 2009). It grows abundantly in southeastern Asia in moist and sunny situations (Lattoo et al., 2008). It is an erect annual herb with a dark-green quadrangular stem; lanceolate and pinnate leaves; small and white flowers; linear-oblong capsules; and tiny yellowish-brown seeds (Jiang et al., 2009). A huge lack of information relating to the salt-tolerance threshold and modality of mineral nutrition of this herb at saline area is obviously sensible. Moreover, the salinity effects on growth and yield, has direct economic implications. On the other hand, *A. paniculata* is categorized in the group of sensitive plants to salinity. The symptoms of such sensitivity appear by scorched leaf tips and margins, leaf curling, and in extreme cases reduced growth, abscission of leaves, and death of the plant.

However, reports on macro and micronutrients uptake rates are available within plants, but the results have not always been consistently reliable. The relationship between salt tolerance and Macro-micro elements tendency of the plant is not investigated yet. Determination of salinity effects on macro-micro elements and the interrelationships among traits will help to make a better comprehension of growing inquiries and the mechanisms of salt tolerance of the plant under salinity. The present exploration is sort of a first attempt to understand the adaptive features of the quantitative effects of salinity levels on Macro-micronutrients flow in *A. paniculata*.

Results

Assessment of morphological traits before applying salinity stress

The results of our study showed that there were no significant differences among 12 accessions for SL, NL, TDW and chlorophyll content. The low coefficient of variation (CV = 4.1-10.1 %) before applying salinity stress complied with the accuracy of the experiment and homogeneous feedback of the studied accessions (Table 1).

Evaluation of different accessions of A. Paniculata at control conditions

The ANOVA analysis of the investigated characteristics under control condition revealed a significant difference among the studied accessions in terms of all Macro and micronutrients uptake, except for the Fe and Mn nutrients. It also showed that there is no significant difference in total dry weight of the 12 accessions (Table 2). This outcome, in fact evidenced that the selected accessions for this experiment were significantly different from each other in the uptake capacity of microelements even under normal condition.

Effects of different salinity levels on yield and STI

The salinity levels affected the yield of *A. paniculata*. Variation due to salinity levels (S), accessions (A) and their interaction were highly significant ($p<0.01$). The interaction of S × A was not significant in terms of total dry weight (TDW), salt tolerance index (STI) (Table 3). Total dry weight results showed that growth was negatively correlated to the substrate concentration of NaCl ($p<0.01$). Plants grown at the low levels of NaCl (control) reached relatively to the higher total dry weights and did not exhibit the toxicity symptoms. However, the total dry weight was significantly reduced at higher levels of salinity (16 dsm^{-1}), in which the symptoms of salt toxicity was revealed as growth depression. The leaves commenced to fall on the day 20^{th} of applying 16 dsm^{-1} of salinity. The concentration of 16 dsm^{-1} NaCl significantly reduced the dry weight by 29.49%, compared to control. Among the accessions, the highest STI (68.7%) was observed in accession 11264, whereas the lowest (49.1%) with the same condition belonged to accession 11329 (Fig. 1). At high salinity level, the salt tolerance index of tolerant accession (11264) was found to be higher (43.19%) than the sensitive one (11329) with only 18.23%. The greatest decrease in STI was observed at 16 dsm^{-1} of salt stress.

Effects of different salinity levels on macronutrients

The analysis of variance revealed a significant difference between salinity levels, in terms of macronutrients accumulation in the plants. Variation due to the salinity levels (S), accessions (A) and their interaction were highly significant ($p<0.01$). The interaction of S × A was not significant in terms of calcium content (Ca^{2+}) (Table 3). The salinity levels significantly decreased all macronutrient contents (Fig. 2 and Fig. 3). The salinity levels significantly caused changes in performances of all accessions. All accessions showed a decreased in K^+ content under salt treatment. The potassium content of tolerant accession was found to be higher than sensitive one in the salt medium. Moreover, the calcium (Ca^{2+}) accumulation of different accessions decreased under salt treatment. The saline treatment also decreased the other macronutrients (P, N and Mg^{2+}) in all accessions compared with control. The decreases in tolerant accessions were lower than sensitive accessions. The potassium contents of accession 11264 and 11179 were found to be higher than accession 11329 and 11306 in the salted medium. The high salinity level of 16 dsm^{-1} significantly reduced the K^+ content by 81.8 %, compared to control. The phosphor, nitrogen, calcium and magnesium contents of tolerant accession(11264) were found to be higher than the sensitive one(11329) under high level of salinity (Table 4).

Effects of different salinity levels on micronutrients

The salinity levels significantly affected all accessions in terms of Na^+ and Zn content, while there were no significant differences among accessions based on Cu, Fe and Mn (Table 5). The interaction of S × A was not significant in terms of Fe concentration. The salinity levels significantly increased micronutrients except Mn (Fig. 2 and Fig. 4). Among the

Table 1. Analysis of variance on measured characteristics of 12 accessions of *A. paniculata* before salinity stress at mature stage.

Source	df	Mean Square			
		NL	SL	TDW	CHLO
R	2	10.50*	12.35^{ns}	0.75*	0.51^{ns}
Accession	11	0.48^{ns}	3.16^{ns}	0.22^{ns}	2.07^{ns}
Error	22	0.54	4.56	0.11	4.51
CV%		4.8	8.7	10.1	4.1

* and ns, refer to 5% and not significant, respectively. NL: number of leave, SL: shoot length, RL: root length, TDW: total dry weight and CHLO: chlorophyll.

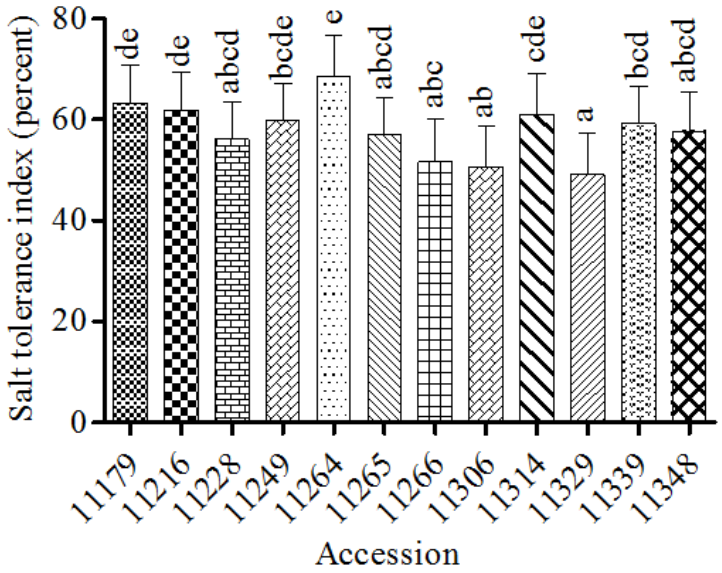

Fig 1. The effects of salinity levels on salt tolerance index in 12 acessions of *A. paniculata.* Error bars show approximately 99% confidence intervals and different letters indicate significant difference among accessions using Duncan's multiple comparison test at $P < 0.01$.

accessions, the highest Na^+ accumulation (7.03%) was observed in accession 11329, whereas the lowest Na^+ accumulation (4.44%) belonged to accession 11264 both at same condition (Fig. 2). In all accessions Na^+, Cu, Zn and Fe content increased under the effect of salt treatment, while Mn content decreased at the same condition (Table 4). In the high salinity level, some accessions such as 11264 and 11149 showed significantly less Na^+ than other accessions (11329 and 11306).

Correlation among the traits under salinity stress condition

The correlation among most of the measured traits was significant at $p<0.01$ (Table 6 and Fig. 5). Interestingly, Na^+ concentration was highly correlated to STI, while others not. The correlation between STI and Na^+ as well as Zn and Fe concentrations were negatively significant, while a positive correlation was determined between STI and K^+, Ca^{2+}, Mg^{2+}, N and Mn concentrations under salt stress. There was no significant correlation between STI and P and Cu content.

Cluster analysis

The cluster analysis of the 12 accessions of *A. paniculata* using UPGMA-Ward method in control conditions produced two clusters for STI and all macro and micronutrients (Fig. 6), while under salinity conditions four clusters created for STI and all nutrients (Fig. 7). Cluster 1 (red color) contained three accessions including a tolerant accession, cluster 2 (green color) contained three accessions, cluster 3 (blue color) contained three accessions including a sensitive accession and cluster 4 included three accessions. The distance between leader and joiner accession in cluster analysis was 4.45 based on all measured traits. According to cluster analysis, the studied accessions reacted in different ways under control and salt conditions, where number of clusters in control was two and then increased up to four clusters under salt stress. In fact, the change in number of clusters happened due to the salt treatment.

Discussion

After 30 days of treatment, the results indicated that applied NaCl inhibited the plant growth, and led to a decrease in dry material in the salted medium compared to control. This may be related to the effect of salt stress, which causes the limitation of water absorption and biochemical processes. A linear decline in dry material correlated with increasing salinity levels was recorded in our experiment. This result was in agreement with many other reports on the other plants, in which the dry weight under salt-stress was less in the control medium (Paek et al., 1988; Liu and Van Staden, 2000; Munns, 2002; Parida and Das, 2005). Sensitive accessions under salinity condition produced less dry weight than tolerant accessions. For instant, accessions 11264 (tolerant) produced 31.3% less dry weight in high salinity level, while the sensitive accessions such as 11329 produced 50.9 % less dry weight compared to control. Several

Table 2. Analysis of variance on nutrient characteristics of 12 accessions of *A. paniculata* in control condition.

Source	df	Mean square										
		TDW	K^+	P	N	Ca^{2+}	Mg^{2+}	Na^+	Cu	Zn	Fe	Mn
Accession	11	22.4^{ns}	0.21^{**}	0.008^{**}	0.58^{**}	0.05^{*}	0.005^{**}	0.02^{**}	24.8^{**}	493.3^{*}	4074.7^{ns}	588.6^{ns}
Error	22	10.7	0.05	0.001	0.13	0.02	0.001	0.004	5.9	156.5	2632.1	469.6
CV%		10.1	9.2	12.7	7.2	24.2	11.3	21.8	25.8	18.0	39.2	32.5

Table 3. Analysis of variance on yield, STI and macronutrients in 12 accessions of *A. paniculata* under salinity stress.

Source	df	Mean square						
		TDW	STI	K^+	P	N	Ca^{2+}	Mg^{2+}
Salinity (S)	4	3179.4**	30474.4**	1.59**	0.025**	6.85**	0.24**	0.042**
Accession (A)	11	50.9**	473.9**	0.12**	0.009**	0.52**	0.08*	0.004**
S×A	44	10.9ns	96.9ns	0.12**	0.005**	0.44**	0.05ns	0.003**
Error	109	10.6	130.2	0.05	0.002	0.21	0.03	0.002

**, * and ns, refer to 1%, 5% and not significant, respectively. TDW: total dry weight and STI: salt tolerance index

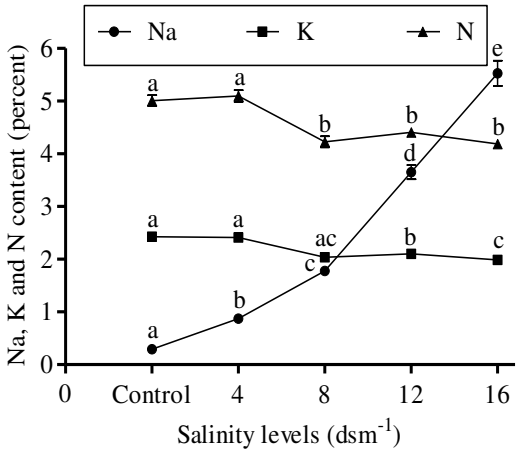

Fig 2. The effects of salinity levels on trend of Na^+ and K^+ accumulation of *A. paniculata.* Vertical bars represent S.E. for three samples and different letters indicate significant difference among the salinity levels using Duncan's multiple comparison test at $P < 0.01$.

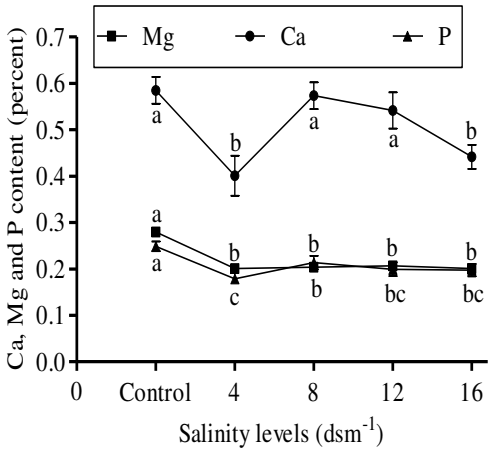

Fig 3. The effects of salinity levels on macro nutrients accumulation in *A. paniculata.* Vertical bars represent S.E. for three samples and different letters indicate significant difference among the salinity levels using Duncan's multiple comparison test at $P < 0.01$.

researchers, have reported similar differences between sensitive and tolerant genotypes in different plant species (Munns, 2002).

The K^+ is required for cells to maintain the osmotic balance, opening and closing of stomata and an essential co-factor for many enzymes (Yeo, 1998). High stomata K^+ requirement is reported for photosynthesis (Chow et al., 1990). The result of the present study showed that NaCl treatment caused an increase in Na^+ concentration, and a decrease in K^+ and Ca^{2+} concentration in all accessions. Seemingly, this result is similar to findings of the other researchers (Colmer et al., 2005; Maatuis and Amtmann, 1999; Munns et al., 2002). However, the increase in Na^+ concentration in sensitive accession like 11329 was higher than tolerant accession such as 11264, but the decrease in K^+ and Ca^{2+} concentration in sensitive was higher than the tolerant accession.

Calcium is important during salt stress, e.g. in cell wall and membranes, and regulates growth and development of plant (Hepler, 2005). As a result, Ca^{2+} fertilizers may reduce Na^+ toxicity to the plants. Besides, the role of Mg^{2+} in chlorophyll structure and as an enzyme co-factor is considerable. Another importance of Mg^{2+} in plants is in the export of photosynthetic, which leads to improve chlorophyll reduction in Mg^{2+} deficient source leaves (Marschner and Cakmak, 1989). In general, salinity reduces N content in plants (Feigin, 1985). The interaction between salinity and P is very complex and there is no clear mechanism to explan the changes of P uptake in response to salinity stress in different species (Grattan and Grieve, 1992). However, it is known that P concentration is related to the photosynthesis rate (Overlach et al., 1993) and; therefore, decrease of P in leaves will reduce the plant growth.

In saline soils, the solubility of micronutrients (Fe, Zn, Mn, and Cu) is mainly low, and plants grown in these soils often develop deficiencies in these nutrients (Page et al., 1990). Differences can be attributed to plant type, plant tissue, salinity level and environmental conditions. Thus, salinity stress either promotes or inhibits the uptake of some micro nutrients depends on plant species. The Fe, Zn and Cu content increase in response to rising NaCl levels, which is consistent with other researchers that reported high uptake of these micronutrients (Moreno et al., 2000; Alam, 1999). In present study, we found a similar tendency of increased concentrations of all micronutrients. Nevertheless, the Mn was an exceptional case in this approach. Manganese is important in activity of enzymes involved in respiration and synthesis.

Table 4. The effects of salinity levels on macro and micronutrients in 12 accessions of *A. paniculata.*

Accession	K^+%	P%	N%	Ca^{2+}%	Mg^{2+}%	Na^+ %	Zn (ppm)
11179	2.3±0.08 ^{bc}	0.17±0.02 ^{a}	4.7±0.17 ^{bcd}	0.61±0.04 ^{c}	0.24±0.01 ^{cd}	2.0±0.4 ^{a}	93.1±5.0 ^{c}
11216	2.2±0.05 ^{ab}	0.18±0.02 ^{ab}	4.4±0.15 ^{abc}	0.57±0.07 ^{bc}	0.23±0.01 ^{bcd}	2.4±0.5 ^{abcd}	78.1±2.6 ^{ab}
11228	2.2±0.06 ^{ab}	0.21±0.02 ^{bcde}	4.4±0.12 ^{abc}	0.39±0.05 ^{a}	0.19±0.01 ^{a}	2.5±0.5 ^{abcd}	72.9±3.0 ^{ab}
11249	2.2±0.06 ^{abc}	0.20±0.02 ^{abcd}	4.6±0.19 ^{bcd}	0.45±0.05 ^{ab}	0.20±0.01 ^{ab}	2.2±0.4 ^{ab}	76.6±4.0 ^{ab}
11264	2.1±0.06 ^{ab}	0.19±0.02 ^{abc}	4.3±0.16 ^{ab}	0.53±0.05 ^{abc}	0.22±0.01 ^{bcd}	2.3±0.4 ^{abc}	73.2±3.1 ^{ab}
11265	2.2±0.08 ^{ab}	0.20±0.02 ^{abc}	4.7±0.18 ^{bcd}	0.43±0.06 ^{ab}	0.20±0.02 ^{abc}	2.1±0.4 ^{ab}	80.4±4.5 ^{b}
11266	2.2±0.08 ^{ab}	0.20±0.02 ^{abcd}	4.6±0.15 ^{bcd}	0.55±0.05 ^{abc}	0.23±0.01 ^{cd}	2.4±0.6 ^{abc}	78.5±4.3 ^{b}
11306	2.2±0.11 ^{ab}	0.25±0.02 ^{f}	4.7±0.24 ^{bcd}	0.44±0.05 ^{ab}	0.21±0.01 ^{abc}	2.4±0.6 ^{abc}	66.6±3.6 ^{a}
11314	2.4±0.13 ^{c}	0.23±0.01 ^{cdef}	4.8±0.21 ^{cd}	0.50±0.04 ^{abc}	0.22±0.02 ^{abc}	2.5±0.6 ^{bcd}	75.7±5.6 ^{ab}
11329	2.1±0.09 ^{ab}	0.24±0.02 ^{ef}	4.6±0.16 ^{bcd}	0.54±0.05 ^{abc}	0.22±0.01 ^{abc}	2.9±0.7 ^{d}	77.2±4.6 ^{ab}
11339	2.1±0.09 ^{a}	0.20±0.02 ^{abcd}	4.2±0.18 ^{a}	0.63±0.06 ^{c}	0.25±0.01 ^{a}	2.8±0.7 ^{cd}	78.6±6.2 ^{b}
11348	2.2±0.07 ^{ab}	0.23±0.02 ^{def}	4.8±0.13 ^{d}	0.48±0.07 ^{abc}	0.21±0.01 ^{abc}	2.8±0.7 ^{cd}	83.3±5.6 ^{bc}

Different letters indicate significant difference between the values of pairs of treatment within columns (mean values ± standard error) at P < 0.01 according Duncan's multiple comparisons test.

Table 5. Analysis of variance on micronutrients in *A. paniculata* under salinity stress.

Source	df	Mean square				
		Na^+	Cu	Zn	Fe	Mn
Salinity (S)	4	166.69**	203.46**	1285.33**	42579.03*	3217.18**
Accession (A)	11	1.21**	47.06ns	614.52**	4263.75ns	159.87ns
S× A	44	0.73**	68.28**	352.13**	4402.89ns	527.14**
Error	109	0.38	26.53	195.66	4434.5	266.70

**, * and ns, refer to 1%, 5% and not significant, respectively.

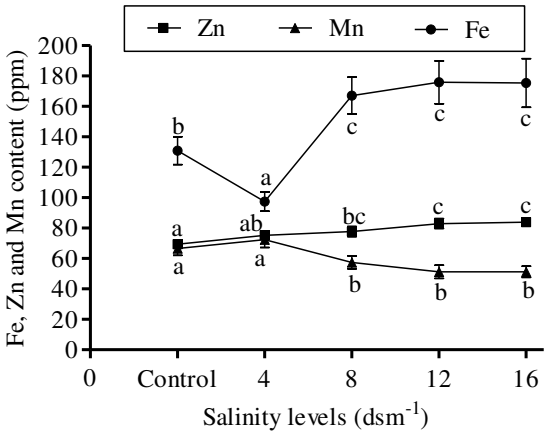

Fig 4. The effects of salinity levels on micronutrients accumulation in *A. paniculata.* Vertical bars represent S.E. for three samples and different letters indicate significant difference among the salinity levels using Duncan's multiple comparison test at P < 0.01.

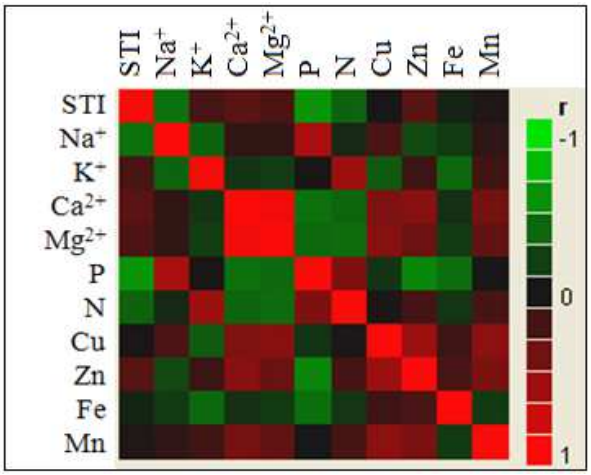

Fig 5. Correlations among 12 accessions for STI, macro and micronutrients under salinity condition. The strength and direction of the correlations among the different traits are indicated by the color (red indicates positive correlations while green indicates negative correlations, and the shading represents the strength of the correlation).

It is difficult to suggest a mechanism to explain the salinity impacts on micronutrients due to the relatively small differences between control and salt-treated plants and the non-linear relationships between some of the micronutrient contents in plants.

Materials and methods

Genetic material and germination conditions

Twelve accessions of *A. paniculata* seeds provided from the Agro Gene Bank, University Putra Malaysia, used for this study during September 2010 - April 2011 (Table 7). The seeds were surface sterilized with 10% sodium hypochlorite (NaOCl) solution for 10 minutes (Talei et al., 2011) and thoroughly rinsed with distil water. Then, seeds were soaked in 15 cm diameter separate Petri dishes containing filter papers No.2 moistened with sterile water. The Petri dishes were sealed with parafilm to prevent any water loss and then incubated under controlled growth chamber (light/dark regime of 14/10h at 28 - 30^{o}C, relative humidity 60- 75%). The seedlings at two leaves stage were transferred into the jiffy media.

Table 6. Phenotypic correlation coefficients (r) among measured traits in 12 accessions of *A. paniculata* under salinity conditions.

	STI	Na^+%	K^+%	Ca^{2+}%	Mg^{2+}%	P%	N%	Cu (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)
STI	1										
Na^+	-.785**	1									
K^+	.478**	-.503**	1								
Ca^{2+}	.206**	-0.097	.202**	1							
Mg^{2+}	.466**	-.291**	.430**	.752**	1						
P	0.107	-.150*	.174*	0.002	0.111	1					
N	.421**	-.435**	.503**	-0.106	0.140	.254**	1				
Cu	-0.143	0.131	-0.030	0.074	0.076	-0.072	-0.081	1			
Zn	-.326**	.233**	-0.031	-0.033	-0.028	0.010	-0.120	.228**	1		
Fe	-.293**	.305**	-.227**	0.078	-0.040	.177*	-0.117	0.078	.347**	1	
Mn	.296**	-.277**	.184*	-.154*	0.088	.277**	.366**	0.037	0.137	0.079	1

The significant correlation is indicated by ** (P<0.01) and * (P<0.05).

Table 7. List of 12 accessions of *A. paniculata*

No.	Accession number	State	Vernacular name
1	11179	Selangor	Tutup Bumi
2	11216	Negeri Sembilan	Hempedu Bumi
3	11228	Negeri Sembilan	Hempedu Bumi
4	11249	Negeri Sembilan	Hempedu Bumi
5	11264	Perak	Akar Cerita
6	11265	Perak	Akar Cerita
7	11266	Perak	Akar Cerita
8	11306	Johor	Hempedu Bumi
9	11314	Terengganu	Lidah Ular
10	11329	Kelantan	Lidah Ular
11	11339	Kelantan	Lidah Ular
12	11348	Terengganu	Lidah Ular

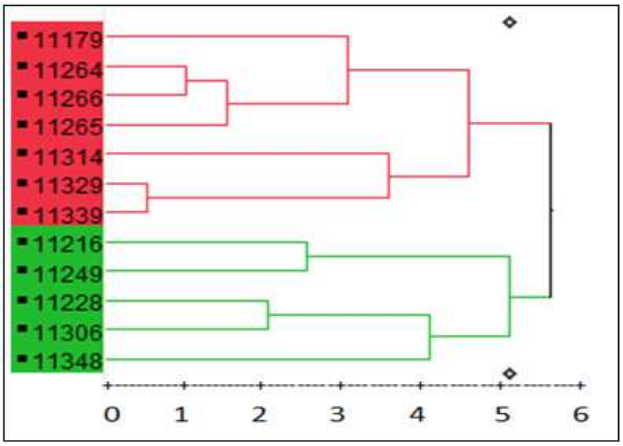

Fig 6. Dendrogram generated by UPGMA clustering method of 12 accessions of *A. paniculata* based on STI, macro and micro-nutrients at control condition.

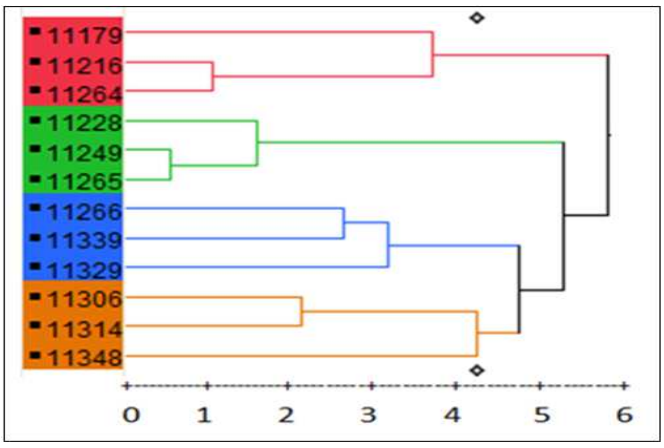

Fig 7. Dendrogram generated by UPGMA clustering method of 12 accessions of *A. paniculata* based on STI, macro and micro-nutrients under salinity condition. Tolerant and sensitive accessions were placed in the red and blue clusters, respectively.

Experimental design

We carried out a split plot experiment based on Randomized Complete Block Design (RCBD) with two factors and three replicates. The factors were five different concentration of saline water [control (0), 4, 8, 12 and 16 dsm^{-1}] in main plots and 12 different accessions in sub main plots.

The 30-day-seedlings were transferred from jiffy media into the pot with sand medium. After 30 days from culturing (almost in 70 days old), the plants were placed in different salinity levels (0, 4, 8, 12 and 16 dsm^{-1}) on Hoagland medium. Each plant was irrigated once a day with five levels of saline water. After every three salinity applications, plants were again irrigated with normal Hoagland nutrient solution. After 30 days salinity exposure, all plants were harvested and data on total dry weight (TDW) (after drying at 68^{o}C for 48 hours), salt tolerance index (STI), Na^+, K^+, Ca^{2+}, Mg^{2+}, N, P, Cu, Zn, Fe and Mn content were measured.

Determination of macro and micronutrients content

Nitrogen was determined by the digestion method described by (AOAC, 1990) using an automated ion analyzer (Lachat instrument, Wisconsin, USA) and others macro and micronutrients content were determined by using an Analyst 5100, Perkin Elmer instrument, USA described by Miller (1998).

Statistical analysis

At first, the raw data were tested for normality using the SAS software No.9 and the main data were then analyzed for measured traits using analysis of variance and the Duncan's multiple range test ($P < 0.01$).

Acknowledgments

This work was supported by University Putra Malaysia (UPM). The macro and micronutrients analysis were carried out in the Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia. We also thank Mr. Jamil Omar for his technical assistance.

References

Alam SM (1999) Nutrient uptake by plants under stress conditions. Handbook of Plant and Crop Stress: 285-313.

AOAC (1990) Official methods of analysis of the Association of Official Analytical Chemists. 15th edition, vol 7. Washington, DC.

Ashraf M and Harris P (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Sci 166(1): 3-16.

Basak A, Banik UK, Basak S, Seiah NG, Li S (2006) Evaluation of Anti-Proprotein Convertase Activity of Diterpene Andrographolid Derived Products. Regulation of carcinogenesis, angiogenesis and metastasis by the proprotein convertases (PCs): a new potential strategy in cancer therapy: 137-154.

Chow W, Ball M, Anderson J (1990) Growth and photosynthetic responses of spinach to salinity: implications of K+ nutrition for salt tolerance. Funct Plant Biol 17 (5):563-578.

Chun JY, Tummala R, Nadiminty N, Lou W, Liu C (2010) Andrographolide, an herbal medicine, inhibits interleukin-6 expression and suppresses prostate cancer cell growth Genes Cancer 1(8): 868-876.

Colmer TD, Munns R, Flowers TJ (2005) Improving salt tolerance of wheat and barley: future prospects. Aust J Exp Agric 45 (11):1425-1444.

Cramer GR, Lynch J, Läuchli A, Epstein E (1987) Influx of Na+, K+, and Ca2+ into roots of salt-stressed cotton seedlings: effects of supplemental Ca2+. Plant Physiol 83(3): 510-516.

Dumrongsak P, Nadege B, Catherine A, Georges M, Alail B, Helene M (2009) Effect of *Andrographis paninculata* extract and Andrographolide on hepatic cyt P450 mRNA expression and monoxygenase activity after in vivo administration to rats and in vitro in rat and human hepatic cultures. Chemico Biol Interaction 179(2-3): 247-255.

FAO (2008) Land resources, management, planning and use. Available online at: http://www.fao.org/ag/agl/agll/spush/ Accessed 25 April.

Feigin A (1985) Fertilization management of crops irrigated with saline water. Plant Soil 89(1): 285-299.

Grattan S and Grieve C (1992) Mineral element acquisition and growth response of plants grown in saline environments. Agric Ecosys Environ 38(4): 275-300.

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Annu Rev Plant Biol 51(1): 463-499.

Hepler PK (2005) Calcium: a central regulator of plant growth and development. Plant Cell Online 17(8): 2142-2155.

Jiang X, Yu P, Jiang J, Zhang Z, Wang Z, Yang Z, Tian Z, Wright SC, Larrick JW, Wang Y (2009) Synthesis and evaluation of antibacterial activities of andrographolide analogues. Europ J Medicin Chem 44(7): 2936-2943.

Ko HC, Wei BL, Chiou WF (2006) The effect of medicinal plants used in Chinese folk medicine on RANTES secretion by virus-infected human epithelial cells. J Ethnopharmacol 107(2): 205-210.

Lattoo SK, Dhar RS, Khan S, Bamotra S, Bhan MK, Dhar AK, Gupta KK (2008) Comparative analysis of genetic diversity using molecular and morphometric markers in *Andrographis paniculata* (Burm. f.) Nees. Genc Res Crop Evol 55(1): 33-43.

Liu T and Van Staden J (2000) Selection and characterization of sodium chloride-tolerant callus of *Glycine max* (L.) Merr cv. Acme. Plant Growth Reg 31(3): 195-207.

Luo Q, Yu B, Liu Y (2005) Differential sensitivity to chloride and sodium ions in seedlings of *Glycine max* and *G. soja* under NaCl stress. J Plant Physiol 162(9): 1003-1012.

Maas E and Grieve C (1987) Sodium-induced calcium deficiency in salt-stressed corn. Plant Cell Environ 10(7): 559-564.

Maatuis FJM and Amtmann A (1999) K+ nutrition and Na+ toxicity: the basis of cellular K+/Na+ ratios. Ann Bot 84(2): 123-133.

Marschner H and Cakmak I (1989) High light intensity enhances chlorosis and necrosis in leaves of zinc, potassium, and magnesium dificient bean (Phaseolus vulgaris) plants. J Plant Physiol 134(3): 308-315.

Miller RO (1998) Nitric-perchloric acid wet digestion in an open vessel. In 'Handbook of Reference Methods for Plant Analysis'.(Ed YP Kalra), CRC Press: Boca Raton, FL, 57-61.

Moreno DA, Pulgar G, Romero L (2000) Yield improvement in zucchini under salt stress: determining micronutrient balance. Scientia Hort 86(3): 175-183.

Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25(2): 239-250.

Munns R, Husain S, Rivelli AR, James RA, Condon AG, Lindsay MP, Lagudah ES, Schachtman DP, Hare RA (2002) Avenues for increasing salt tolerance of crops, and the role of physiologically based selection traits. Plant Soil 247(1): 93-105.

Munns R and Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59: 651-681.

Overlach S, Diekmann W, Raschke K (1993) Phosphate translocator of isolated guard-cell chloroplasts from Pisum sativum L. transports glucose-6-phosphate. Plant Physiol 101(4): 1201-1207.

Paek KY, Chandler SF, Thorpe TA (1988) Physiological effects of Na2SO4 and NaCl on callus cultures of *Brassica campestris* (Chinese cabbage). Physiol Planta 72(1): 160-166.

Page A, Chang A, Adriano D (1990) Deficiencies and toxicities of trace elements. Agric Salinity Assess Manage 71: 138-160.

Parida AK and Das AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Safety 60(3): 324-349.

Serraj R and Sinclair TR (2002) Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant Cell Environ 25(2): 333-341.

Talei D, Mihdzar AK, Khanif MY, Saad MS, Valdiani A (2011) Effects of different surface sterilizers on seed germination and contamination of king of bitters (*Andrographis paniculata* Nees.). Am Eurasian J Agric Environ Sci 10(4): 639-643.

Tozlu I, Moore GA, Guy CL (2000) Effects of increasing NaCl concentration on stem elongation, dry mass production, and macro-and micro-nutrient accumulation in Poncirus trifoliata. Funct Plant Biol 27(1): 35-42.

Valdiani A, Mihdzar AK, Tan SG, Talei D, Puad MA, Nikzad S (2012) Nain-e Havandi (*Andrographis paniculata*) present yesterday, absent today: A plenary review on underutilized herb of Iran's pharmaceutical plants. Mol Biol Rep DOI: 101007/s11033-011-1341-x.

Vallejo AJ, Yanovsky MJ, Botto JF (2010) Germination variation in Arabidopsis thaliana accessions under moderate osmotic and salt stresses. Ann Bot 106(5): 833-842.

Yeo A (1998) Molecular biology of salt tolerance in the context of whole-plant physiology. J Expe Botany 49(323): 915-929.

Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53(1): 247-273.