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Effect of salinity on proximate mineral composition of purslane (*Portulca oleracea* L.)

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Abstract

Purslane (*Portulaca oleracea*) is a drought and salt tolerant annual plant which contains high amounts of beneficial antioxidant vitamins and minerals. The objective of this study was to determine the influence of salt stress on the growth and mineral composition of purslane (*Portulaca oleracea* L.). Four salinity levels namely 0 (control), 66, 132 and 264 mM NaCl were tested. Full grown leaf and stems of purslane samples were harvested at 10 and 20 days of the saline treatment exposure. Growth of purslane plants was more suppressed under 264 mM compared to 132 mM. Salinity levels and planting harvest time significantly influenced the levels of protein, water content and ash. The protein content of purslane leaves decreased with increasing salinity and time of exposure treatment. However, carbohydrates and mineral residue content increased. The highest mineral residue content was found in leaves exposed to the maximum salinity levels. The mineral composition was also affected by salinity levels, Na^+, Mg^{++} and Cl^- uptake and accumulation increased with the increment of salinity. The Ca^{++}, K^+ and Zn^+ ion levels decreased with increasing salinity. Accumulation of Ca^{++} and Zn^+ in leaves was higher while K^+ and Na^+ were higher in the stems. The relative ratio of Na^+/K^+, Na^+/Ca^{++}, Na^+/Mg^{++}, Mg^{++}/Ca^{++} and Mg^{++}/K^+ increased with increasing salinity treatment. The findings of this study revealed that purslane can tolerate moderate salinity levels (66 and 132 mM). Therefore, purslane can be a potential to become a key vegetable crop, especially for functional food and nutraceutical applications.

Keywords: Purslane, Salinity, growth and mineral content.
Abbreviations: mM-Millimole, ANOVA- Analysis of Variance, DW-Dry Weight, LSD- Least Significant Difference, EC-Electrical Conductivity.

Introduction

The 'weed' purslane (*Portulaca oleracea* L.) is gaining special attention by agriculturists and nutritionists. It is a common weed in turfgrass areas as well as field crop areas (Uddin et al., 2009; 2010). Plants need essential mineral elements for their growth and development but the presence of excessive soluble salts in the soil is harmful to the majority of plants. The main factors responsible for soil salinization are the low quality of irrigation waters, excessive fertilization and the deficient drainage of some soils. As a result, the yield of crops decreases and it is no longer possible to grow more salt-sensitive species (Vasconcelos, 1987; Grieve, 2000; Singh and Chatrath, 2001). Soil salinity is the major abiotic stress drastically affecting the plant growth and crop productivity. Due to continuous build up of salinity in the soil, millions of hectares of arable land have now become unfit for cultivation (Ahmad et al., 2007). Now, it is well accepted that salinity can affect the plant growth by changing their morphological, physiological and biochemical as well as anatomical characteristics (Tester and Davenport, 2003). Changes in the lipid composition and oil quality characteristics and antioxidant properties of some oilseed crops as result of abiotic stresses such as salinity and drought have been reported recently (Flagella et al., 2002; Ali et al., 2010).

Salinity is also a problem in Malaysia. Due to anthropogenic contributions to global warming, the rate of sea level is expected to rise and; thus, causing salt-water intrusion into the coastal areas posing threat to major agricultural crops (Vasconcelos, 1987; Aksoy et al., 2003). As a result, the yield of crops will be decreased and it is no longer possible to grow more salt-sensitive species (Vasconcelos, 1987; Grieve, 2000; Singh, and Chatrath, 2001). An approach to solving the salinity problem is shifting towards growing salt-tolerant crops (Shannon et al., 2000). Usually, all plants are affected by salinity leading to general inhibition of growth (Grieve, 2000). Under the prevailing conditions of increasing salinity, it is necessary to grow some salt-tolerant plants, which can withstand increasing stress of salinity and can substitute already existing crops economically. Purslane is naturally a weed plant, which has tremendous medicinal potential and can substitute existing crops. Keeping in view the medicinal importance and its adaptability to highly saline conditions, the present study was planned to investigate the effect of different salt levels on mineral composition of purslane plants.

Results and Discussion

Proximal nutritional composition

The proximal nutritional composition and growth of purslane leaves was significantly affected by the salinity level of the treatment and time of harvest (Table 1). Succulence is an important property of halophytic or saline-tolerant plants because a high number of halophytic plants show some kind of succulence in their tissues. Ottow et al. (2005) reported the development of leaf succulence and osmotic adjustments in the salt-tolerant tree *Populus euphratica* exposed to sodium chloride salinity, from 25 to 400 mM NaCl. The C_4 dicotyledonous *P. oleracea* possesses succulent leaves with branched venation and is composed of various types of cells (Lara et al., 2003). In this study, mean water content of purslane leaves slightly decreased when the salinity level of the treatment was raised (Table 1). After 10 days of treatment (harvest 1), there was a reduction in mean water content with increasing salinity. These data suggest that, although the plants were exposed to saline stress, they were able to maintain the water levels in the leaves. Higher temperatures may have led to higher levels of transpiration in purslane leaves, which might explain the decrease in the total water content in the plants.

Many studies refer that an increase in salinity levels in a plant's surrounding environment leads to a decrease of nitrogen uptake and accumulation by the root (Neumann, 1997; Nilsen and Orcutt, 2000). In many plants, protein synthesis is affected by the exposure of the plant to sodium chloride, and in some cases, protein hydrolysis occurs with the release and accumulation of free amino acids in the tissues (Neumann, 1997; Nilsen and Orcutt 2000; Levit 1980). Mean total protein in purslane leaves ranged from 8.81% to 18.56% Dw (Table 1). The protein levels in purslane cultures (control plants) were similar to or higher than those of other forage or vegetable food crops.

These high crude protein values were also reported by Ezekwe et al. (1999) and Obied et al. (2003) and placed purslane above alfalfa, which has a crude protein content of 17% DW, and is currently the most important commercial vegetable crop in the USA. The highest mean total protein content was present in control plants and the lowest in plants exposed to the extreme saline treatment, 264 mM.

Many of the protein synthesis steps are very sensitive to alterations in the ionic equilibrium in the cells, which may result in a complete blockage of protein metabolism (Singh and Chatrath, 2001; Neumann, 1997; Levit, 1980). These data suggest that, although the protein biosynthesis was adversely affected by the salinity, the plant ability to synthesize the protein was not totally compromised by the salinity levels used. Furthermore, the increase in uptake and accumulation of chloride ions (Cl^-) in plant tissue generally results in the decrease in nitrate ($NO3^-$) accumulation in the plant's aerial parts (Lara et al., 2003; Nilsen and Orcutt, 2000; Rieger and Litvin, 1998). In this study, lower amounts of mean total protein were found in purslane leaves, where higher levels of chloride accumulation were observed (Table 2). The accumulation of organic compounds, such as sugars and amino acids in the cytoplasm plays an important role in the osmotic balance of plants (Morgan, 1992). Mean total carbohydrate content in purslane leaves was determined in relation to the other nutrients, by subtracting the values (in percentage) of total protein, total lipid and ash contents and included soluble sugars and fibers. In the control plants, the total carbohydrate content ranged from 61% to 67% (Table 1), which was higher than that reported by Obied et al. (2003). Higher amounts of total carbohydrates in purslane leaves were observed in plants exposed to the highest salinity level (24 dS m^{-1}) compared to control samples in both experiments, at both harvests ($P < 0.05$). A slight increase in total carbohydrates was also observed in plants exposed to 132 mM but was not significantly different from control plants. For moderate salinity conditions, a decrease in total carbohydrate content was observed relative to control plants (Table 1). An increase in carbohydrates content, because of saline treatment, has also been measured in other plants. Kafi et al. (2003) reported an increase in total carbohydrate content in leaves of wheat cultivars with increasing salt content in the medium (NaCl 300 mM).

Mineral composition

The mineral composition of purslane leaves and stems were significantly ($P < 0.05$) affected by the salinity level and duration of salt treatment with some interesting trends. Sodium and Cl^- contents increased with increasing levels of salinity as was expected; however, the rate of increase was drastically higher in stem than in leaf (Table 2, 3). In control plants, Na^+ contents were about 4.0 and 4.75 m mg^{-1} DW in leaves (Tabel 3) and stems, respectively. Salt treatment for 10 and 20 days duration increased Na^+ contents to 1.5- to 2-fold in leaves, and up to 4 –fold in stems depending on the salinity levels. Chlorine contents in leaves also increased up to about 2.5-fold in leaves and ~3.5-fold in stem depending on salinity levels and duration of salt exposure. Magnesium contents also slightly increased with increasing levels of salinity; however, rate of increases were similar in leaf and stem.

Among other mineral components, Ca^{++} contents were about 2.5 folds higher in leaves compared to stems in control plants. The K^+ contents were 2 folds lower in leaves compared to stem in control plants. Salt treatments gradually reduced Ca^{++} (13-46% in leaf and 15-47% in stem) and K^+ contents (7-22% in leaf and 2-22% in stem) depending on salinity levels and duration of salt exposure. Zinc contents also gradually decreased both in leaves and stems depending upon the salinity levels and duration of exposure. However, Fe^{++} contents gradually decreased in leaves with salinity levels and duration of exposure and increased in stems as the duration of exposure increased.

Ions can interact with the soil and the plant in different ways, which can lead to deficiency or toxicity phenomena that affect growth and development (Nilsen and Orcutt, 2000; Zhu, 2003). The ionic uptake by the cell is affected by the environmental salinity, which affects the relative availability of the ions in the area surrounding the root (Nilsen and Orcutt, 2000; Grattan and Grieve, 1999). The mineral composition of purslane leaves and stems were significantly ($P < 0.05$) affected by the salinity level of the treatment. In this study, salinity significantly affected the ion concentration and distribution within the purslane leaves (Table 2) and stems (Table 3). The ion uptake and accumulation in the leaves of purslane plants was lower, with the exception of Ca^{++} and K^+, with higher values in this season. The same was observed for Fe^{++} and Zn^{++} ion accumulation in stems, but some variation in Ca^{++}, Mg^{++} and K^+ ion accumulation was observed. Stem Ca^{++} and Mg^{++} mean concentrations were lower in the spring test after 10 days of treatment. Stem K^+ values were higher in after 10 days of treatment and lowered after 20 days. This variation may be related to the ion movement and preferential accumulation in the plant tissues. With increasing salinity, the plants need more Ca^{++}.

Table 1. Proximal nutritional composition of purslane leaves (*P. oleracae*) at different salinity levels.

Harvest days	Salinity (EC mM)	Nutrient content (% DW)			
		Total protein	Ash	Total carbohydrates	Water content
10	0	16.3a±0.6	10.5b±0.6	67.2b±1.9	91.6a±0.4
	66	12.4b±0.9	11.8ab±0.7	69.1ab±0.4	90.4ab±0.4
	132	9.2c±0.3	12.0ab±0.6	72.1ab±0.4	88.1b±1.1
	264	8.8c±0.5	12.7a±0.6	72.3a±0.8	87.5c±0.9
20	0	18.5a±0.9	11.0c±0.5	61.6a±1.7	92.3a±0.3
	66	12.8b±0.7	12.8bc±0.7	59.7a±2.8	91.6ab±0.6
	132	11.7b±0.6	14.3b±0.9	62.0a±0.9	89.5b±0.8
	264	9.5c±0.4	18.5a±0.9	65.8a±1.6	84.5c±0.9

Table 2. Mineral concentration of purslane leaves (*P. oleracae*) at different salinity levels.

Harvest days	Salinity (EC mM)	Mineral concentration (m mg^{-1} DW)						
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Fe^{++}	Zn^{++}	Cl^{-}
10	0	15.4a±0.7	18.5c±0.5	3.7c±0.1	10.3a±0.4	1.7a±0.1	1.2a±0.03	0.88a±0.04
	66	13.3b±0.4	17.5d±0.3	5.3b±0.1	10.0a±0.4	1.5b±0.1	1.1a±0.03	1.8b±0.07
	132	10.3c±0.6	20.2b±0.7	7.3a±0.4	8.5a±0.4	1.2c±0.04	0.8b±0.06	1.9b±0.07
	264	8.4d±0.8	21.1a±0.5	7.5a±0.4	8.4a±0.4	1.0d±0.05	0.7b±0.66	2.4c±0.27
20	0	17.4a±0.3	17.3c±0.7	4.2c±0.2	9.5a±0.3	1.64±0.06	1.3a±0.08	0.95a±0.04
	66	14.9b±0.6	16.5d±0.7	5.8b±0.3	9.3a±0.3	1.41±0.07	1.1b±0.04	1.8b±0.05
	132	11.4c±0.4	19.2b±0.5	8.1a±0.3	8.0ab±0.4	1.18±0.04	0.9c±0.04	2.0b±0.04
	264	9.2d±0.3	20.1a±0.6	8.2a±0.2	7.4b±0.3	0.95±0.05	0.8c±0.03	2.5c±0.09

Simultaneously, the transport of this ion can be reduced by ionic interactions, precipitation and the higher ionic strength of the medium, which leads to a reduction of the Ca^{++} availability for the plant (Nilsen and Orcutt, 2000; Grattan and Grieve, 1999). When Ca^{++} and Mg^{++} concentrations in the medium fall below the critical limits of the plant cell, the K^{+} uptake also decreases (Grattan and Grieve 1999). In this study, the increase of NaCl concentration in the irrigation solution resulted in a decrease in the accumulation of Zn^{++}, Ca^{++} and K^{+} in plant tissues, as well as an increase in Na^{+} and Cl^{-} concentrations.

A slight increase in the Mg^{++} concentration was observed in leaves and stems of purslane plants exposed to higher levels of saline treatment, 132 and 264 mM (Tables 2 and 3). The effect of the saline treatments on Fe accumulation was not clear. Leaf Fe^{++} concentration decreased with the increase of salinity in the irrigation water. Stem Fe^{++} also decreased with increasing salinity after 10 days of treatment exposure, but after 20 days, the stem Fe values were higher for the plants exposed to higher levels of saline treatment. Stem Fe^{++} concentration from plants exposed to 132 mM treatment did not differ.

The environmental salinity affects not only the nutrient uptake but also the distribution and accumulation in the plant tissues. In a medium, where the Na^{+} ions are at a higher concentration, a reduction on the uptake and mobility of calcium ions to the growing areas will affect the vegetative and reproductive organs of the plant (Singh, and Chatrath, 2001, Grattan and Grieve 1999). In this study, we observed a differential accumulation of the Na^{+}, K^{+}, Ca^{++} and Mg^{++} in plant organs as seen by Grieve and Suarez (1997), with a preferential accumulation of Ca^{++} in the leaves and Na^{+} and K^{+} in the stems. Apparently, the increase in Na^{+} uptake and accumulation led to a reduction of Ca^{++} accumulation in the purslane leaves and stems. At all salinity levels, stem Ca^{++} was roughly 30% of that in the leaves (Tables 2 and 3) and decreased with the increase in salinity in the irrigation solution. Although a preferential accumulation of Mg^{++} in purslane leaves, as previously reported by Grieve & Suarez (1997), was not observed here, but this ion was present in significant amounts in purslane tissues. Ca^{++} and K^{+} deficiencies induced by the increase in salinity may be responsible for the reduction in plant growth and yield seen in the highest saline treatment (264 mM). High concentrations of sodium and chloride ions in soil or irrigation solutions may reduce the uptake and accumulation of ions and nutrients, leading to extreme ratios of Na^{+}/ Ca^{++}, Na^{+}/ K^{+}, Ca^{++}/ Mg^{++} and Cl^{-}/NO3 (Grieve & Suarez, 1997). In high salinity conditions, high concentration of Na^{+} not only interferes in K^{+} uptake at root level but also causes alterations in the integrity and selectivity of membranes.

As salinity increased, purslane leaves and stems Ca^{++} and K^{+} concentrations decreased, while Na^{+} and Mg^{++} concentrations increased (Tables 2 and 3). The increased Na^{+} with the concomitant decreased the K^{+} in plant. This might be attributed to the competition and resultant selective uptake between K^{+} and Na^{+}, which causes increase in the uptake of Na^{+} at the cost of K (Kuiper, 1984; Uddin et al., 2011; Uddin and Juraimi, 2012; Uddin et al., 2012a; Uddin et al., 2012b). To verify some ionic interactions, Na^{+}/ Ca^{++}, Mg^{++}/Ca^{++}, Na^{+}/K^{+} and Mg^{++}/K^{+} ratios were calculated for plants exposed to 20 days of saline treatment (harvest 2). Gorham (1994) reported that at lower external salinities (<100 mM NaCl), the enhanced K^{+}/Na^{+} discrimination character of wheat cultivars resulted in a low leaf Na^{+} concentration. In addition, the maintenance of K^{+} concentration at saline condition is not similar to those in plants grown at normal condition (without salt).

Both Na^{+}/ K^{+} and Mg^{++}/ K^{+} in leaves and stems increased as the K^{+} and Mg^{++} concentrations remained constant while salinity increased (Table 4). In highest level of salinity, leaf Na^{+}/K^{+} and Mg^{++}/K^{+} ratios increased 6.5-fold and 1.5-fold, respectively, in relation to control. Stems Na^{+}/ K^{+} ratio increased about 5-fold and there was only marginal increase in Mg^{++}/K ratio. Leaf Na^{+}/ K^{+} and Mg^{++}/K^{+} ratios increased 4- and 2-fold, respectively. Stems Na^{+}/K^{+} and Mg^{++}/K^{+} ratios increased 5- and 2-fold, respectively (Table 4). Mg^{++}/Ca^{++} ratios, higher than one, led to a reduction of the growth and yield of cereals such as wheat and sesame (Gorham, 1994; Yahya, 1998). In this study, Mg^{++}/Ca^{++} ratios were always

Table 3. Mineral concentration of purslane stems (*P. oleracae*) at different salinity levels.

Harvest days	Treatment (EC mM)	Mineral concentration (m mg^{-1} DW)						
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Fe^{++}	Zn^{++}	Cl^{-}
10	0	6.0a±0.18	17.0a±0.48	4.4c±0.46	20.2a±0.50	2.1a±0.15	1.1a±0.04	0.8b±0.03
	66	5.0a±0.34	17.9a±0.23	5..7b±0.30	17.7b±0.65	1.6b±0.05	0.9a±0.06	1.5a±0.10
	132	4.0a±0.19	19.5b±0.50	7.6a±0.23	16.3b±0.32	1.3bc±0.06	0.6b±0.04	1.5a±0.19
	264	2.86b±0.12	20.6b±0.36	8.1a±0.23	14.4c±0.43	1.2c±0.06	0.5c±0.04	1.8a±0.21
20	0	6.1a±0.39	16.0c±0.32	5.0d±0.36	17.4a±0.66	0.9b±0.08	0.7a±0.04	1.7c±0.05
	66	5.2b±0.14	16.8c±0.38	6..7c±0.41	15.5b±0.36	0.8b±0.06	0.6a±0.07	3.1b±0.46
	132	4.4b±0.25	18.3b±0.63	17.7b±0.30	14.9b±0.61	1.2a±0.07	0.5ab±0.05	4.0b±0.45
	264	3.1c±0.13	20.1a±0.44	19.8a±0.57	13.9b±0.56	1.3a±0.06	0.4b±0.04	5.9a±0.18

Table 4. Ion selectivity ration of purslane leaves and stems (*P. oleracae*) at different salinity levels.

Harvest days	Salinity (EC mM)	Ion selectivity ratio				
		Na^{+}/ K^{+}	$Na^{+}/C\ a^{++}$	Na^{+}/ Mg^{++}	Mg^{++} / Ca^{++}	Mg^{++} / K^{+}
Leaves	0	0.36c±0.01	0.24d±0.01	0.20c±0.01	1.2c±0.02	1.7b±0.02
	66	0.53b±0.01	0.40c±0.01	0.30b±0.02	1.3c±0.01	1.7b±0.04
	132	0.84a±0.01	0.71b±0.01	0.36a±0.01	1.9b±0.05	2.3a±0.12
	264	0.89a±0.01	0.90a±0.04	0.35a±0.01	2.5a±0.19	2.5a±0.07
Stem	0	0.21d±0.02	0.74d±0.07	0.26c±0.02	2.8c±0.10	0.8d±0.03
	66	0.32c±0.02	1.1c±0.08	0.32bc±0.02	3.5c±0.34	0.9c±0.03
	132	0.46b±0.01	1.8b±0.14	0.38ab±0.01	1.9b±0.05	1.1b±0.04
	264	0.56a±0.02	2.8a±0.16	0.39a±0.01	7.2a±0.38	1.4a±0.04

greater than unity, at all salinity levels, both in the leaves and stems, but a reduction of the growth and yield of purslane cultures was only seen in the highest level of salinity.

Materials and methods

Plant materials and treatments

Two-week-old seedlings of *Portulaca oleracea* L. (yellow flower, red stem) were transplanted into pots (500 cm^3) filled with the same soil mixture and placed in a glasshouse, Faculty of Agriculture, Universiti Putra Malaysia. The soil media was prepared by thoroughly mixing washed river sand (<2 mm diameter) and peat grow ($KOSAS^R$) in the ratio of 9: 1 (v/v). The prepared soil media was pulverized and inert materials, visible insect pest and plant propagules were removed. The soil media was dried at room temperature and thoroughly mixed for analysis. All pots were fertilized with NPK Green (15:15:15) at 50 kg N ha^{-1}. The amount of fertilizer per pot was 513 mg and applied fortnightly. The pot area was 0.0154 m^2 (14 cm diameter). Everyday fresh water was applied to grow the plant properly. Each pot contained four seedlings. Six weeks after the seedlings were transplanted, four saline treatments were imposed with irrigation solutions, so that the Electrical Conductivity (EC) mean values for the four treatments were: 0.0 (control), 66, 132 and 264 mM NaCl. The salinity treatment (NaCl) applied until 3 weeks. Air temperatures in the glasshouse ranged from 25 ^{0}C to 35 ^{0}C. Relative humidity ranged from 21% to 81%. On 10 and 20 days after the completion of salinization (hereafter designated as harvest 1 and 2), three plants were cut at soil level and weighed. Each plant was picked at random from each pot and in each harvest from the same pot.

Experimental Design

Pots were arranged in a randomized block design with three blocks (replicates) of treatment combinations. Plant yield, proximal nutritional composition and mineral content were subjected to an analysis of variance (ANOVA) using procedure of the SAS Statistical Software (SAS 2004). Treatment means were compared using LSD Test.

Plant tissue chemical analysis

The individual samples of plant material were separated into leaves and stems. Chemical composition (Table 1) was determined only on leaves, and mineral content was determined in both leaves and stems according to Miller, 1998. The resultant flour was placed in airtight glass jars and stored at -20 ^{0}C until analysis. The flour from each sample was analyzed for ash, moisture, crude proteins and oil using the Association of official Analytical Chemist (AOAC 1990) methods. The percentage of total carbohydrate content in purslane leaves was determined by subtraction of the values of the other nutrients, protein, and ash fractions and also includes soluble sugars and fibers. Results are the mean of triplicate determinations and expressed as percentage on a dry matter basis.

Protein content analysis

Protein content was determined according to a semi-automated FOSFA official method (1982). A sample of leaf was digested for 10 min with a digestion mixture of sulfuric acid/hydrogen peroxide/potassium sulfate, with selenium dioxide as a catalyst. The final end point in the ammonia titration was measured photometrically. Fiber content was determined according to the ISO method (1981). A 2.5 g leaf sample of finely ground was weighed and freed from fat by extraction with 15 mL of *n*-hexane. The test portion was boiled with a sulfuric acid solution (0.255 mol L^{-1}), followed by separation and washing of the insoluble residue. The residue was then boiled with sodium hydroxide (0.313 mol L^{-1}), followed by separation, washing, and drying. The dried residue was weighed and ashed in a muffle furnace (EYELA, TMF-2100, Tokyo, Japan) at 600 °C, and the loss in mass was determined. Ash content was determined according to the ISO method (ISO, 1977). Two grams of the test portion was taken and carbonized by heating on a gas flame. The carbonized material was then ashed in an electric muffle furnace (EYELA,

TMF-2100, Tokyo, Japan) at 550 °C until constant mass was achieved.

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