Biochemical and Growth Responses of *Moringa peregrina* (Forssk.) Fiori to Different Sources and Levels of Salinity

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Abstract

Background: **As *Moringa peregrina* is a valuable medicinal plant in traditional medicine, it is necessary to determine responses of this plant to salinity.**

Objective: **To determine some biochemical and growth responses of *Moringa peregrina* to salinity at the seedling stage.**

Methods: **This experiment was conducted in Institute of Medicinal Plants-ACECR, on base of factorial experiment as completely randomized design with four replications. The treatments included a combination of 3 different sources of salt (NaCl, NaCl + $CaCl_2$ and natural saline water) and eight levels of salinity (control, 2, 4, 6, 8, 10, 12 and 14 dS/m).**

Results: **The results showed that the salinity levels had significant effect ($p < 0.01$) on the studied traits. Although the sources of salinity hadn't a significant effect on shoot and root length, other traits were significantly ($p < 0.01$) affected by it. This study indicated that *Moringa peregrina* hadn't reduction in growth parameters and seedling emergence up to 6 dS/m, then these traits significantly decreased with increasing salinity. Proline and carbohydrate content as compatible organic solutes increased with increasing salinity and these results indicated an important role of proline and carbohydrates in *Moringa peregrina* tolerance to salinity.**

Conclusion: **The survival and no reduction in seedling emergence and growth parameters up to 6 dS/m indicated that moringa was a salt tolerant species at the early growth stage.**

Keywords: ***Moringa peregrina*, Carbohydrate, Proline, Seedling emergence, Salinity**

Introduction

Soil salinity is a major environmental stress that drastically affects crop productivity. It is estimated that every year more than one million hectares of land are subjected to salinization [1]. The seeds of halophytes and non-halophytes plants respond in a similar way to salinity stress, e.g. the initial germination process is delayed under salt stress [2, 3].

Salt stress tolerance in plants is a complex phenomenon that may involve developmental changes as well as physiological and biochemical processes [4, 5]. Accumulation of compatible organic solutes, so called osmolytes, in leaves is also a common response to salt stress in several plants. Soluble carbohydrates, amino acids, organic acids, proline and betaines are some of the most common compatible organic solutes found in these plants [6 - 8]. Proline accumulation is one of the adaptation mechanisms of plants to salinity and water deficit [9, 10]. It has also been widely advocated that proline accumulation uses as parameter of selection for salt stress tolerance [11]. Proline concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants [12, 13].

Moringa is an important food commodity as all plant parts such as leaves, flowers, fruits, and immature pods can be used as a highly nutritive vegetable. These are commonly consumed in India, Pakistan, Philippines, Thailand, Hawaii and many parts of Africa [14, 15]. Recently, this plant is cultivated in the south of Iran. The leaves are of highly nutritious, rich in vitamins A and C and act as a good source of natural antioxidants [16, 17]. In addition, Moringa is believed to have multiple medicinal uses. For example, the barks, roots, leaves and flowers of Moringa tree are used in traditional medicine and folk remedies in many countries [16, 17]. The seeds also contain high quality oil that can be used in cooking, cosmetics and lubrication [14, 18].

Although Moringa, *Moringa peregrina* (Forssk.) Fiori is one of the medicinal plants and its cultivation is continuously being extended in the world including Iran, no information is available on the response of this plant to salinity. On the other hand, early growth stages is one of the most critical phases of plant life [19, 20], the present study was carried out to explore the effects of salinity on Moringa at seedling emergence stage.

Material and Methods

In this experiment, the seeds exposed to three different sources of salinity (NaCl solution, NaCl + $CaCl_2$ solution and natural saline water), and each source included eight levels of salinity (control, 20 dS/m, 4 dS/m, 6 dS/m, 8 dS/m, 10 dS/m, 12 dS/m and 14 dS/m) in a factorial experiment as completely randomized design with four replications. Natural saline water was obtained from Hoz-e-Soltan Lake in Qom, Iran.

This study was conducted in Institute of Medicinal Plants-ACECR, Iran, in winter 2010. Since the purpose of this study was the investigation of salinity condition on seedling emergence of Moringa and its seeds had big size; they were sown in sand instead of petri dish for better absorption of salinity. Ten seeds were sown in every pot which was filled with sand. The pots were irrigated with related saline solutions. For preserving saline water and inhibiting the evaporation, the pots were covered with plastic bags, and then they were maintained in growth chamber with 23°C and 58% humidity, 16 hours light and 8 hours dark. The seedlings were emerged at the 8th day. Every day the seedlings were counted and the seedling emergence percentage was measured. At the 18th day, the pots were moved to lab for next measurements including the length of shoot and root, fresh and dry weight of shoot and root, proline content of shoot and root, and carbohydrates content of shoot and root. For determining of dry weight of shoot and root, these parts were dried at 70°C for 48 hours (until there was no decrease in weight). The proline content was determined as described by Bates et al., 1973 [21]. Soluble carbohydrates were determined according to method of Dubois et al., 1956 [22].

The data were analyzed using SAS software and the mean comparisons were done following least significant different at $p \leq 0.05$.

Results

The results showed that salinity levels had significant effect ($p<0.01$) on all traits (Table 1). The **s**eedling dry weight, seedling emergence percentage, root length and shoot length hadn't significantly decreased by salinity levels up to 6 dS/m. Also, the proline and carbohydrate of shoot and root significantly increased with increasing salinity levels (Table 2).

Although, the salinity reduced seedlings emergence percentage, the reduction in seedling emergence wasn't significant up to 6 dS/m of salinity level. The seedlings emergence was about 71.7 percentages in non-saline treatment, declined to about 45% when salinity increased up to 8 dS/m. On the other hand, the salinity level that seedling emergence started to decrease was 8 dS/m and the amount of this reduction was 25%. In addition, with increasing salinity level from 10 to 14 dS/m, no more reduction in seedling emergence was observed (Table 2).

Although, the sources of salinity hadn't significant effect on shoot and root length, the dry and fresh weight, proline and carbohydrate content, and seedling emergence percentage were significantly affected by sources of salinity (Table 1).

The highest salinity level decreased the dry weight, root and shoot length about 80%, 47% and 63%, respectively. Obviously, the proline and carbohydrate content of shoot and root increased more than 65% in 14 dS/m salinity level in comparison with control. Interestingly, the proline content of root was higher than the shoot at different salinity levels. Unlike to proline, the content of soluble carbohydrates in the shoot was higher than the root at different salinity treatments (Table 2).

According to table 1, the effect of salinity sources was significant on all of the parameters except root and shoot length. However, the minimum negative effect was related to $NaCl + CaCl_2$ solution and the highest amounts of seedling emergence and growth was observed in this source (Table 3).

Table 1- Analysis of variance for different parameters of *Moringa peregrina*

Sources of Variation	d.f.	Mean square				
		Seedling dry weight	Seedling fresh weight	Seedling emergence percentage	Root length	Shoot length
Sources of salinity	2	0.03941726**	0.75769101**	0.14349799**	0.41815072ns	0.06904772ns
Levels of salinity	7	0.16463619**	1.70393363**	0.66062302**	3.04454688**	0.26753912**
Salt * source	14	0.00691773ns	0.03864134ns	0.03265447ns	0.27348975ns	0.01658195ns
Error	72	0.0049407	0.0558791	0.0190568	0.1979778	0.0223647

Table 1- continued

Sources of Variation	d.f.	Mean square			
		Shoot proline	Root proline	Shoot carbohydrate	Root carbohydrate
Sources of salinity	2	0.16062117**	0.4736203**	7126.8493**	2750.8698**
Levels of salinity	7	0.29054772**	0.63207102**	19110.9096**	21026.2273**
Salt * source	14	0.07263815ns	0.04730688ns	553.9769ns	1527.6062ns
Error	72	0.00002137	0.00000348	0.7093	0.7836

ns: nonsignificant
**: significant at 0.01 probability levels

Table 2- Effect of salinity levels on the studied parameters of *Moringa peregrina*

Salinity levels (dS/m)	Mean				
	seedling dry weight (gr)	Seedling fresh weight (gr)	Seedling emergence percentage	Root Length (cm)	Shoot length (cm)
Control	0.72167 a	3.1917 ab	71.667 a	12.008 ab	9.4250 a
2 dS/m	0.73167 a	3.8000 a	70.000 a	13.167 a	8.4167 a
4 dS/m	0.55333 ab	3.0558 ab	60.000 a	13.0833 a	8.833 a
6 dS/m	0.69083 ab	2.9825 b	60.000 a	12.500 a	8.6667 a
8 dS/m	0.30167 c	1.6833 c	45.000 b	9.917 b	6.2500 b
10 dS/m	0.12917 d	0.7725 d	21.667 c	6.000 c	5.0000 bc
12 dS/m	0.13750 d	0.7475 d	22.500 c	6.667 c	4.3333 c
14 dS/m	0.11500 d	0.6233 d	25.000 c	6.417 c	3.5000 c

Table 2- continued

Salinity levels (dS/m)	Mean			
	Shoot proline (µmol/gr)	Root proline (µmol/gr)	Shoot carbohydrate (mg ml $^{-1}$)	Root carbohydrate (mg ml $^{-1}$)
Control	0.387803g	0.4191178h	42.6415g	45.7572h
2 dS/m	0.391392g	0.4389061g	61.8631f	52.0876g
4 dS/m	0.399864f	0.4605668f	85.7867e	56.6293f
6 dS/m	0.418084e	0.5140349e	101.0350d	97.2741e
8 dS/m	0.502252d	0.6871133d	114.6842c	111.5908d
10 dS/m	0.528473c	0.7424986c	131.0317b	131.8325c
12 dS/m	0.636325b	0.9346766b	151.0000a	137.2367b
14 dS/m	0.831840a	1.0042534a	151.1133a	151.3583a

Within column means followed by the same letter (a …) are not significantly different at the 0.05 level, according to L.S.D. test

Table 3- Effect of different sources of salinity on the studied parameters of *Moringa peregrina*

Sources of Salinity	Mean				
	Seedling dry weight (gr)	Seedling fresh weight (gr)	Seedling emergence percentage	Root Length (cm)	Shoot length (cm)
NaCl	0.37250 b	1.7488 b	41.875 b	9.3469 a	6.15 4b
NaCl + CaCl2	0.52313 a	2.7566 a	53.125 a	10.5933 a	7.5938 a
Water of Ghom river	0.37219 b	1.8159 b	45.938 b	9.9688 a	6.6563 ab

Table 3- continued

Sources of Salinity	Mean			
	Shoot proline (µmol/gr)	Root proline (µmol/gr)	Shoot carbohydrate (mg ml $^{-1}$)	Root carbohydrate (mg ml $^{-1}$)
NaCl	0.532205 b	0.7728058a	111.7808b	87.3705 c
NaCl + CaCl2	0.570558 a	0.5295071c	115.1315a	101.9710b
Natural saline water	0.433250 c	0.6481248 b	87.7711c	104.5709a

Within column means followed by the same letter (a …) are not significantly different at the 0.05 level, according to L.S.D. test

Discussion

The seedling emergence is the most critical period for a crop subjected to salinity [19, 20]. Seed germination of plants has been reported to decline with increasing salinity levels [23, 24, 25]. This study indicated that the seedling emergence had no reduction up to 6 dS/m and then decreased with increasing salinity. Also, percentage of seedling emergence was about 25% at salinity level of 14 dS/m. Therefore, this plant can reasonably tolerate salinity up to 6 dS/m and emerged until 14 dS/m.

The proline is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses [26 - 32]. As table 2 showed, salinity levels hadn't significant effect up to 6 dS/m on all studied traits except proline and carbohydrate content. The proline and carbohydrate content of shoot and root significantly increased in company with increasing salinity. So it can be concluded that accumulation of proline and carbohydrate can be order to salinity tolerance. For this reason, Moringa could act in salinity levels up to 6 dS/m like non-saline condition and survived in 14 dS/m salinity level.

The results indicated that the proline content was much greater in root than in the shoot. Higher levels of proline in roots compared with shoots may be due to the fact that the roots are the primary sites of water absorption and must maintain osmotic balance between water absorbing root cells and external media [33, 34]. Therefore, the enhancement of proline content in root is a valuable strategy for plant to survive at saline condition.

Accumulation of proline under environmental stress in many plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants. For example, while in salt-tolerant alfalfa plants proline concentration in the root rapidly doubled under salt stress, in salt-sensitive plants the response was slow [12]. Accordingly, it can be suggested that Moringa may be known as a salt tolerant plant.

Since NaCl + $CaCl_2$ source had the minimum negative effect on seedling emergence and growth (Table 3), it can be guessed that calcium has an important role in plant preservation from salinity. Experimental evidence implicates Ca^{2+} function in salt adaptation. Externally supplied Ca^{2+} reduces the toxic effects of NaCl, presumably by facilitating higher K^+/Na^+ selectivity [35].

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