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Effect of drought and salinity stresses on mineral and total protein contents of *Moringa*

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Abstract

Abiotic stresses such as drought and salinity severely affect the mineral nutrients and protein level of plants. Current study was conducted on two *Moringa* species *M.oleifera* and *M.peregrina* inside glass house, using three factorial arrangement in Randomized complete Block Design (RCBD) to investigate how different levels of drought (2, 7 and 14 days) and salinity (0, 10, 25, 35, 45 and 60%) impact the level of Nitrogen (N), Phosphorus (P), Potassium (K), Iron (Fe) and total proteins in the leaves of *Moringa* species. A dynamic decline in both nutrients and proteins contents were reported in both species with increasing interval of drought and concentrations of salinity. Most remarkable decline for both nutrient (N, P, K and Fe) and total proteins in both species were reported at drought interval of fourteen days and salinity concentration of sixty percent. The current study concluded that abiotic stresses such as drought and salinity significantly hampered the uptake of important nutrients in addition to metabolic activities involved in the synthesis of proteins.

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Introduction

Both *Moringa oleifera* and *M. peregrina* are native species of south Asia that were brought in other parts of the world where they acclimatized due to multiple use and medicinal values as well as environmental importance (El-Alfy *et al.,* 2011). In Saudi Arabia, *Moringa*is mainly distributed in South and North Hijaz (Hegazi, 2015). Drought and salinity unanimously disrupt the mineral-nutrient relation in plants by impacting the transport, availability and assimilation of nutrient in plants (Frosi *et al.*, 2017). Moreover, saline stress also provokes the deficiency of ions or imbalance due competition of nutrient entities like K^+, Ca^{2+}, and NO^{-3} with toxic ionic entities Na^+ and Cl^- (Bartels and Sunka, 2005). Mineral elements exhibit a dynamic function in regulating the resistance of plants to drought or salinity. In fact both drought and salinity has parallel impact on the growth of plant via water deficit, therefore K^+ is equally worthy to retain the turgor pressure of the plant under either condition of stress (Blumwald, 2000). Besides the high K^+: Na^+ ratios are also important to determine the resistance of the plant to salinity (Carden *et al.*, 2003). On the other hand the Ca^{2+} is considered as main signaling factor for determining the resistance of plant to both drought and salinity; hence extensive focus is directed now days to elucidate the interaction between Ca^{2+} and each stress (Cramer, 2002). The rise in uptake of Nitrogen (N) and Phosphorus (P) uptake by plants is supposed as more fundamental under drought conditions as compared to salinity (Frosi *et al.*, 2017). Besides, iron (Fe) is an important constituent involved in synthesis of enzymes involved in metabolic activities and protein synthesis (Brumbarova *et al.*, 2008). Drought and salinity have direct effect on the distribution, uptake and assimilation of Fe that indirectly hampered metabolic activities and the synthesis of proteins (Tripathi *et al.*, 2018). Deposition of Na^+ in leaf is responsible for reduction in photosynthesis as well as inhibition of uptake of essential minerals like N, P and Potassium (K) (Al-Karaki, 2000). Under saline conditions, the competition between Cl^- and NO^{-3} illustrates the significant role of N in determining the growth of salinity affected plants (Carden *et al.*, 2003). As compared to N, P, and K other micronutrients are less important in imparting plants resistance to drought and salinity (Bartels and Sunka, 2005). In general the supply of nutrients to the plants in soil during drought and salinity stress can combat the adverse of effects of these stresses on plants. However, this supplementation cannot make improvement in the growth of plants when nutrients are present in soil in sufficient quantities but the salinity and drought stresses are more intense. Therefore, current study was conducted to understand how salinity and drought impact the distribution of important nutrients like N, P, K, Fe and total proteins in the leave of *Moringa*.

Materials and methods

Current study was conducted on two species of *Moringa*, *oleifera* and *peregrina* for estimating the impacts of different intervals (2, 7 and 14 days) of drought and levels (0, 10, 25, 35, 45 and 60%) of salinity on the nutrients (N, P, K and Fe) and total proteins contents. The experiment was conducted in three factorial arrangement using Randomized complete Block Design (RCBD), with species as factor A, drought as factor B and salinity as factor C.

Drying and grinding of samples

The leaves samples taken from *Moringa* species were dried at 65°C until a constant weight was reached. All the dried leaf samples were powdered using a colloidal Grinder and stored at room temperature until digestion.

Digestion of plant samples

The dried and grind leaf (0.5 g) samples were digested as indicated by Jakson (1973). Then volume of digest was made up to 50 mL by adding distilled water. After making volume it was filtered and used for the determination of mineral elements (N, P and K) according to standard protocols.

Determination of N, P and K in Moringa

Nitrogen from the leaf digest was determined by Kjeldahl method using Automatic Kjeldahl

Analyzer, while P was measured by spectrophotometer at 410 nm wavelength. N and P were expressed as percentage (%) of dry weight of leaves. The percentage of K from the leaves of *Moringa* species was determined by using flame photometry technique.

Determination of total protein contents (%)

Protein contents (%) in the leaves were determined by multiplying the value of % N in the leaves with 6.25 (N% × 6.25).

Determination of iron contents (%)

The iron content of *Moringa* species were determined by using the protocol followed by Elgailani and Ishak (2015).Five grams of dried plant parts were converted into powder form after weighing precisely. Afterward approximately 37.5 ml of HNO_3 (conc.) was added and the mixture was kept at room temperature until initiation of reaction. Then samples were heated on a sand bath till the cessation of the generation of brown NO_2 fume. The solution was cooled and followed by the addition of 7.5 ml of 60% perchloric acid. The whole mixture was heated continuously until the evaporation of approximately 50% volume. After this the addition of 10 ml of H_2SO_4 and HNO_3 mixture (1:1) was done and heating was done until the appearance of a pale yellow or clear solution. Following this the solution was cooled and filtration was done in a 100ml flask. The final volume of flask was adjusted by the addition of deionized water. Finally the absorbance of solution was recorded with the help of atomic absorption spectrometer at 248.3nm to determine the iron content.

Statistical analysis

Analysis of variance was done using the statistical software Statistx8.1, and LSD was calculated at ($P \leq 0.05$).

Results

Effect on Fe percentage

The varying levels of salinity treatments significantly ($P \leq 0.01$) changed the percentage of Fe content in the leaves of *Moringa* (Table 1). A remarkable decline in iron content was noticed with increasing levels of salinity; however60% salinity depicted the lowest mean content as illustrated in Table 1.

Table 1. Effect of drought, sea water concentrations and *Moringa* species on minerals and total protein contents of Moringa leaves.

Treatments	Fe	K	N	P	TP
Days					
D_1 (2)		1.53a	4.47a	0.36a	22.58a
D_2 (7)		1.34b	3.90b	0.29b	21.72b
D_3 (14)	ns	1.07c	3.62c	0.30b	187.58c
LSD		0.13	0.08	0.02	0.10
SE		0.05	0.04	0.01	0.05
Variety					
V_1(*M. oleifera*)		1.35a	3.64b	0.31a	20.67b
V_2(*M. peregrina*)	ns	1.28b	4.35a	0.32a	21.26a
LSD		0.01	0.26	0.02	0.01
SE		0.01	0.06	0.01	9.48
Salinity					
S_5 (60%)	0.04^{c}	0.90e	3.08d	0.20f	17.67f
S_4 (45%)	0.05^{c}	1.18d	3.5c	0.25e	19.67e
S_3 (35%)	0.05^{c}	1.29cd	3.67c	0.28d	20.78c
S_2 (25 %)	0.11^{ab}	1.40bc	4.03b	0.35c	20.33d
S_1 (10 %)	0.08^{b}	1.48ab	4.79a	0.38b	23b
S_0 (0 %)	0.14^{a}	1.61a	4.90a	0.43a	24.33a
LSD	0.05	0.15	0.18	0.02	0.13
SE	0.03	0.08	0.09	0.01	0.06
Significance					

D	ns	**	**	**	**
V	ns	**	**	ns	**
S	**	**	**	**	**
D× V	ns	ns	**	ns	**
D × S	*	**	**	**	**
V × S	ns	*	**	**	**
D × V × S	ns	*	**	**	**

TP= Total protein

Means followed by the same letter (s) in each column and treatment showed no significant difference

*, ** indicate significant differences at 0.05, 0.01 probability levels respectively while 'ns' indicate non-significant difference.

However, no significant (P≤ 0.05)impact of drought intervals and varietal type was reported on the percentage of Fe. Besides individual treatments, among all two way interactions, only the interaction between drought and salinity significantly (P≤ 0.05) affected the Fe percentage in leaves. For all drought treatments maximum decline in iron percentage was reported at salinity level of 60, as shown in Table 2. No significant affect of three way interaction between drought, variety and salinity was noticed.

Table 2. Effect of interaction between drought and salinity on mineral and total protein contents of Moringa leaves.

Drought(D) (days)	Salinity %	Fe	K	N	P	TP
D1(2)	S0	0.12	1.84	5.40	0.50	26
	S1	0.10	1.65	5.30	0.45	24
	S2	0.08	1.60	4.40	0.40	23
	S3	0.06	1.45	3.85	0.30	22
	S4	0.05	1.35	3.90	0.30	21
	S5	0.05	1.30	3.95	0.20	18
D2(7)	S0	0.23	1.75	4.30	0.42	25
	S1	0.08	1.45	4.80	0.33	25
	S2	0.07	1.45	3.90	0.33	19
	S3	0.05	1.35	3.70	0.28	22
	S4	0.06	1.30	3.80	0.20	20
	S5	0.04	0.78	2.90	0.18	18
D3(14)	S0	0.09	1.25	5.00	0.38	21
	S1	0.05	1.35	4.28	0.35	20
	S2	0.18	1.20	3.80	0.32	18
	S3	0.05	1.08	3.47	0.27	17
	S4	0.05	0.90	2.80	0.24	17
	S5	0.04	0.65	2.40	0.22	16
	LSD	0.01	0.12	0.11	0.06	1.05
	SE	0.03	0.09	0.11	0.01	0.08

TP=Total protein

Means having difference greater than LSD are significant at $P \leq 0.05$.

Effect on Potassium content

All treatments significantly (P≤ 0.01) affected the mean percentage of K in the leaves (Table 1). Mean comparison between different levels of drought revealed high decline in K content by D3compared to D2 and D1. Among species, *M. oleifera* illustrated statistically lower K content as compared to other *M. peregrina*. A prominent reduction in K level was reported for the highest level of (S5) salinity, as compared to other concentrations. Besides the

individual treatments, significant (P≤ 0.01) effect of two way interactions between drought, varieties and salinity were noticed on K content. Besides the effect of individual treatments significant effect of interactions between drought and salinity (D × S), and variety and salinity (V × S) was reported on K content. For interaction D ×S, the highest level of salinity depicted maximum decline in K level for all drought treatments as illustrated in Table 2. The effect of salinity treatment became more prominent with extended intervals of drought treatment. On the other hand for interaction V × S, both cultivars depicted maximum decline in K at the highest concentration of salinity as illustrated in Table 3. In general both species were equally responsive to the increasing concentration of salinity. No effect of three way interaction was observed.

Table 3. Effect of interaction between variety and salinity on mineral and total protein contents of Moringa leaves.

Variety(D) (days)	Salinity %	Fe	K	N	P	TP
V1	S0	ns	1.63	4.77	0.45	24
	S1		1.47	4.28	0.33	22
	S2		1.47	3.70	0.37	21.67
	S3		1.22	3.36	0.28	21
	S4		1.37	3.03	0.23	18.67
	S5		0.97	2.70	0.18	16.67
V2	S0		1.60	5.03	0.42	24.67
	S1		1.50	5.30	0.42	24
	S2		1.33	4.36	0.33	19
	S3		1.37	3.98	0.28	20.56
	S4		1.0	3.97	0.26	20.67
	S5		0.85	3.46	0.21	18.67
	LSD		0.46	1.56	0.09	2.86
	SE		0.08	0.09	0.01	0.06

V1=*M. oleifera*, V2= *M. peregrina*, TP=Total protein

Means having difference greater than LSD are significant at $P \leq 0.05$.

Effect on Nitrogen content

All treatments significantly (P≤ 0.01) affected the value of N content in the leaves of *Moringa* (Table 1)**.** Statistically significant decline in leaf nitrogen content was reported with increasing interval of drought. Among species, *M. oleifera* revealed more decline in N content as compared to *M. peregrine*. A dramatic decrease in N amount was documented for the maximum level of (S5) salinity, as compared to other levels. Besides individual effect of treatments, significant (P≤ 0.01)of all two way interactions was observed on percentage nitrogen content of leaves. For interaction D × S, the lowest value of nitrogen content was recorded for S5 (Table 2), however this decline was more remarkable at D3. Moreover, for interaction V × S, all species showed decrease in N content with increasing levels of salinity, whereas this decline was more dramatic for V1 as compared to V2 as shown in table 3. For interaction V × D, all species demonstrated reduction in the level of nitrogen with increasing interval of drought, however this reduction was more dramatic for D3 (Table 4). No significant (P≤ 0.05) impact of three way interaction between drought, variety and salinity was reported on the value of N content.

Effect on Phosphorus percentage

Both drought and salinity treatment significantly (P≤

0.01) altered the value of P in leaves; however no significant (P≤ 0.05) effect of varietal type was reported on the mean content of P (Table 1). All drought treatments showed statistically significant effect on the level of P in leaves, with maximum decline shown by D3 and D2 as compared to D1. Likewise, the increasing level of salinity significantly reduced the quantity of P in leaves, with maximum reduction was reported for S5. Besides individual effect significant (P≤ 0.01) impact of interactions D × S and V × S was reported for the mean content of phosphorus in laves. For interaction D x S, the maximum decrease in P content was revealed by S5 at all levels of drought, however this reduction was more dramatic at D3 (Table 2). Likewise, for interaction V × S both species illustrated maximum decline at S5 concentration, whereas, this decline was more dramatic for V2 as compared to V1 (Table 3). On the other hand no effect of three way interactions was reported.

Table 4. Effect of interaction between variety and Drought on mineral and total protein contents of Moringa leaves.

Variety (D) (days)	Drought day	Fe	K	N	P	TP
V1	D1			4.35		21.17
	D2			3.52		22.50
	D3			3.05		18.33
V2	D1			4.58		24
	D2	ns	ns	4.28	ns	21
	D3			4.20		18.83
	LSD			0.14		2.50
	SE			0.04		0.05

V1= *M. oleifera* , V2=*M. peregrina*, TP= Total protein

Means having difference greater than LSD are significant at $P \leq 0.05$.

Total Protein content

All factors significantly (P≤ 0.01) affected the quantity of total protein in *Moringa* (Table 1). Mean comparison revealed that protein level significantly decreased with the extension of drought treatments; however more dramatic reduction was noticed for D3 as compared to D2 and D1. Among species significantly less total protein content was recorded in *M. oleifera* (V1) as compared to *M. peregrina* (V2). Likewise, increment in levels of salinity dramatically decreased the mean protein content, whereas the maximum reduction was reported for S5 as compared to all other levels. Besides individual effect, significant (P≤ 0.05) effect of all two ways interactions between the treatments was reported on the level of proteins. For interaction D × S, all drought treatments depicted the lowest value of total proteins at S5 level of salinity as demonstrated in Table 2. This reduction was more prominent for all levels of salinity at drought interval D3. Correspondingly for interaction V × S, both species showed the least value of protein at S5 treatment, however this reduction was more dramatic for V1 at all levels of salinity as compared to V2 (Table 3). Similarly for interaction V × D, both cultivars depicted the lowest value of total protein at D3 level of drought, likewise this reduction was more remarkable for V1 as compared to V2 (Table 4). No significant (P≤ 0.05) effect of three way interaction between drought, varietal type and salinity was noticed on total protein content.

Discussion

The current study was conducted to interrogate the effect of drought treatments, varietal type and salinity levels on the percentage of N, P, K, Fe and Total proteins in the leaves of two *Moringa* species, *M. oleifera and M. peregrina*. Significant decline in the

contents of these parameters was reported with varietal type and increasing levels of drought and salinity. In fact, high level of Na+ and drought creates hindrance in the uptake of some essential nutrient elements such as N, P and K that ultimately creates nutritional imbalance inside the plants (Al-Karaki, 2000). Hu and Schmidhalter (2005) documented that the uptake of N by the plants decreased under saline and drought conditions, probably due to reduction in the activity of N fixing bacteria and disruption in nitrogen assimilation processes. Analogous results were obtained in current study where dynamic reduction in N contents was reported in both species of *Moringa* with increasing levels of drought and salinity. Likewise, it is generally considered that even the mild conditions of drought reduce the uptake of P in plants (Pinkerton and Simpson, 1986). In current study, this was the most probable reason of systematic decline in percentage of P with increasing periods of drought. Besides *Liebersbach et al.* (2004) reported that salinity reduces the availability of P to plants because of ionic-strength effect that declines the P activity. Moreover, in soil solution the concentration of Pis strongly controlled by the process of absorption and less solubility of Ca-P minerals as reported by *Grattan* and *rieve*, 1999. On the other hand, Rouphael *et al.* (2012) documented that drought hinders the uptake, transport and distribution of N, P and K in plant tissues. Moreover, drought and salinity stress individually or simultaneously decrease the uptake of mineral nutrients in plants by declining the supply of nutrients through the process of mineralization and by reducing the diffusion and mass flow from the soil (Schimel *et al.*, 2007; Sanaullah *et al.*, 2012; Lambers *et al.*, 2008; He and Diskstra, 2014).These statements logically justify the outcomes of current study. Apart from this, Feis also considered as an important micronutrient playing important role in agriculture. In plants, it play vital functions from chlorophyll synthesis to the formation vital proteins involved in energy transfer processes inside the plant system (Brumbarova *et al.*, 2008; Gill and Tuteja, 2011). Increase in alkalinity and dryness of soil make the iron content from the soil unavailable to plants due to its existence in insoluble form (Shao *et al.*, 2007; Tripathi *et al.*, 2018). This was the most probable reason that in current study a decline in Fe content was observed with increasing levels of salinity. As the iron is the basic triggering factor in the synthesis of proteins in addition to metabolic activities. Therefore, reduction in total protein contents in both *Moringa* species under drought and saline conditions can be attributed to reduced activity or unavailability of Fe.

Thus, more research should focus on alternative strategies of increasing plant resistance to drought or salinity, including the use of the genetic potential for conventional breeding and/or molecular technologies to introduce appropriate genes and regulatory systems.

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