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Original Article

Nutrients in Two and Three Cotyledon Seedlings of *Peganum harmala* L. Under Soil Salinity

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Abstract

Key words: *Peganum harmala*, soil salinity, *Fe*-deficiency, three cotyledons.

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The final seed germination of *Peganum harmala* L. was significantly decreased at soil salinity of 2.6, 8.6 and 10.8 DS/m, compared with adequate concentration. *Fe* content in three cotyledon seedlings was significantly correlated with the percentage of final seed germination ($rs = 0.94$, $P = 0.0048$), under different concentrations of soil salinity. Normal nutrient order (in two cotyledon seedlings) changed at 10.8 DS/m of soil salinity. Two cotyledon seedlings adapted in the increasing concentrations of soil salinity better than three cotyledon seedlings. Three cotyledons are associated with iron deficiency and can result of *Fe*-deficiency at least during embryogenesis. The results indicate that the variation in cotyledon number of *P. harmala* L. is related with soil salinity, resulting in at least *Fe* deficiency.

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Introduction

In seed plants, embryogenesis is an important process to produce a new generation. External such as drought, salinity as well as endogenous factors such as phytohormones, proteins, transcription and other substances can affect embryogenesis (Unnikrishnan *et al.*, 1990; Khorolsuren & Jamsran, 2005; Umehara *et al.*, 2007). Previous studies reported that salinity effects on seed germination and early seedling growth (Rahman & Ungar, 1990; Gulzar *et al.*, 2001; Bayuelo-Jimenez *et al.*, 2002; Jamil *et al.*, 2005, 2006; Lombardi & Lupi, 2006; Cordazzo, 2007; Necajeva & Ievenish, 2008; Bybordi & Tabatabaei, 2009; Sivasankaramoorthy *et al.*, 2010; Akbarimoghaddam *et al.*, 2011), and approved that the formation of three cotyledons is related with embryogenesis (Taylor & Mundell, 1999; Al-Hammadi *et al.*, 2003; Conner & Agrawal, 2005).

The purpose of this study was to describe whether the variation in cotyledon number of *Peganum harmala* L. is related with soil salinity.

Materials and Methods

Seeds of *P. harmala* and soil (0-20 cm depth) were sampled in Ekhiin Gol oasis (N43°14'679; E099°00'411; alt. 971 m), on 28 August 2001. *P. harmala* grows in clay-loam soil with pH=7.57, 10.8 DS/m of electrical conductivity and soluble *Ca* is 2460 ppm; *Mg* – 390; *Fe* – 422; *Cu* – 13.26 and *Mn* – 6.21 in soil. Soluble salts in water were extracted and prepared water solutions of different salt concentrations. Seed germination was determined at 25±1°C for 10 days in the seed germinator, without dormancy breaking treatments, using Petri dishes and moist blotter by salt solutions with 2.6, 4.7, 5.7, 6.8, 8.6 and 10.8 DS/m of conductivity. Seedlings for nutrient analysis were sampled in second day after germination and during seed coat attached on seedling, because of endosperm used for seedling growth. *Ca*, *Mg*, *Fe*, *Cu* and *Mn* contents in seedlings were determined using atomic absorption spectrophotometer (AAS), in the Institute of Physics and Technology, Mongolian Academy of Sciences. Dried weight of seedlings was 0.011 g.

Rate of seed germination can indicate salinity tolerance during seed germination (Bayuelo-Jimenez *et al*., 2002). Seeds of *P. harmala* quickly germinated (for 3 days after put in germinator), and then final seed germination were used to evaluate effect of soil salinity during seed germination. Nutrient content in seedlings and final seed germination were compared by Spearman Rank Correlation. Data of seed germination and nutrient content in seedlings under different concentrations of soil salinity were analyzed by Tukey-HSD test, using statistic software JMP 4.0.

Results

Maximum of final seed germination was 88% at 6.8 DS/m, and this concentration can be adequate for seed germination of *P. harmala*, while the minimum was 68% at 10.8 DS/m. The final seed germination was significantly decreased at 2.6, 8.6 and 10.8 DS/m, compared with adequate concentration (Fig. 1).

Cu, *Mn*, *Ca* and *Mg* contents in both two and three cotyledon seedlings did not decrease with increasing or decreasing of salinity concentration, but *Fe* content began to decrease at 8.6 DS/m. At 6.8 and 8.6 DS/m of soil salinity, *Fe* content in two and three cotyledon seedlings was similar, but at 10.8 DS/m, *Fe* content in two cotyledon seedlings was larger than in three cotyledon seedlings. Decreasing content of *Fe* in three cotyledon seedlings was larger than in two cotyledon seedlings with increasing concentration, but it

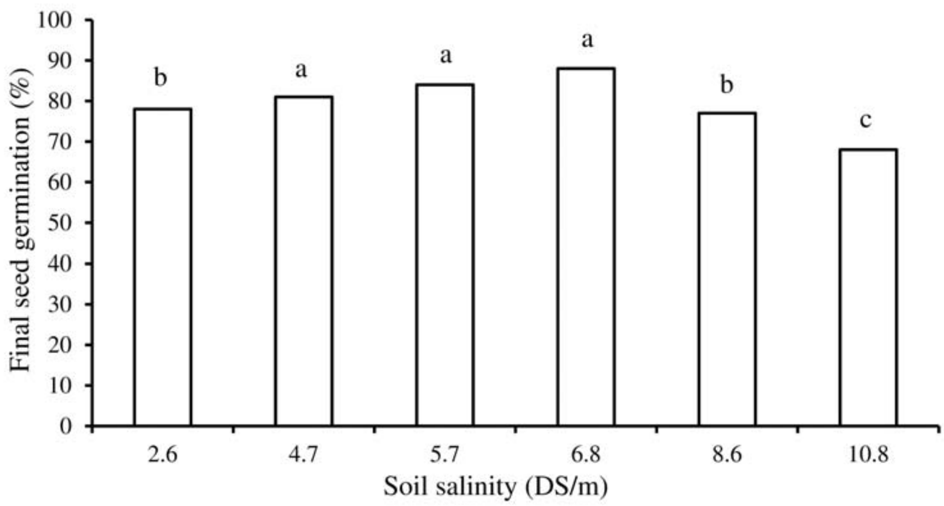

Fig. 1. Final seed germination of *P. harmala* under different concentrations of soil salinity (Tukey HSD test, P<0.05).

Table 1.Comparison of mineral contents between two and three cotyledon seedlings under different concentrations of soil salinity

Soil salinity (DS/m)	*Cu*, ppm		*Mn*		*Fe*		*Ca*		*Mg*	
	Di*	Three**	Di	Three	Di	Three	Di	Three	Di	Three
2.6	87	95	2.6	5	85	173	95	116	244	254
4.7	95	83	3.8	3.9	245	300	125	134	256	259
5.7	101	89	5	3.8	345	336	178	169	265	244
6.8	89	95	5	6.3	327	345	143	161	265	275
8.6	83	77	6.3	6.3	245	218	196	160	281	228
10.8	77	59	6.5	5	218	158	134	166	254	254

* - two cotyledon seedlings, ** - three cotyledon seedlings

was opposite with decreasing concentration, compared with adequate concentration (Table 1). The highest content of *Cu* and *Fe* in two cotyledon seedlings was at 5.7; *Ca* and *Mg* at 8.6, and *Mn* at 10.8 DS/m, whereas that of *Ca* at 5.7 and other at 6.8 DS/m in three cotyledon seedlings.

Nutrient order at 6.8 DS/m in two cotyledon seedlings was different from at 8.6 to10.8 DS/m, whereas that among at 6.8, 8.6 and 10.8 DS/m in three cotyledon seedlings was different. The nutrient order between two and three cotyledon seedlings was similar at 4.7, 5.7, 6.8 and 8.6 DS/m, but that was different at 2.6 and 10.8 DS/m (Table 2).

Discussion

To evaluate salinity effect on seed germination, previous studies used distilled water supply for control and compared with increasing concentrations of salinity (Rahman & Ungar, 1990; Gulzar *et al*., 2001; Jamil *et al*., 2005, 2006; Lombardi & Lupi, 2006; Cordazzo, 2007; Necajeva & Ievenish, 2008; Bybordi & Tabatabaei, 2009; Sivasankaramoorthy *et al*., 2010; Akbarimoghaddam *et al*., 2011), but in the present study we used the adequate concentration of soil salinity for control, because plants do not absorb distilled water in nature.

Final seed germination of *P. harmala* decreases either an increase or decrease of soil salinity concentrations from the adequate concentration, resulting in nutrient absorption difference between two and three cotyledon embryo seeds. Percentage of the final seed germination was the highest when nutrient order in both two and three cotyledon seedlings was similar, like *Fe>Mg>Ca>Cu>Mn* at 6.8 DS/m, while that was lower or lowest when nutrient order between those was different. Normal nutrient order (in two cotyledon seedlings) changed at 10.8 DS/m of soil salinity. At this concentration, seeds with three cotyledon embryo are absorbed nutrients during imbibitions phase, weaker than those with two cotyledon embryo, because of maturation of those embryos was different for embryogenesis. In detail, three cotyledon embryos were immature and two cotyledon embryos were mature. Nutrient order in two cotyledon seedlings was dramatically changed with decreasing concentrations of soil salinity, but that in three cotyledon seedlings was with increasing concentrations, during seed coat attached on seedling. The result indicates two cotyledon seedlings could absorb nutrients and adapt in increasing concentration of soil salinity, stronger than three cotyledon seedlings.

Fe content in both two and three cotyledon seedlings was decreased with increasing concentrations of soil salinity. Whereas, that in three cotyledon seedlings was decreased at 10.8 DS/m, stronger than two cotyledon seedlings, because of three cotyledon seedlings absorbed *Fe* with increasing concentrations of soil salinity, weaker than two cotyledon ones. It means that iron deficiency occurs in seedlings. Three cotyledons are associated with iron deficiency, in addition to auxin accumulation (Al-Hammadi *et al*., 2003).

Fe content in three cotyledon seedlings was significantly correlated with the percentage of final seed germination, under different concentrations of soil salinity (Fig. 2). This correlation suggests that germination of seeds with three cotyledon embryo is depending on *Fe* absorption. On the other hand, final seed germination directly depends on seed maturity for embryogenesis and three cotyledons can result of *Fe*-deficiency at least during embryogenesis. Al-Hammadi *et al*. (2003) reported that the earliest defects of embryo were observed at the transition from the globular to the heart stage of embryogenesis with the

Table 2. Comparison of nutrient orders between two and three cotyledon seedlings under different concentrations of soil salinity

Soil salinity (DS/m)	Two cotyledon seedlings	Three cotyledon seedlings
2.6	*Mg>Ca>Cu>Fe>Mn*	*Mg>Fe>Ca>Cu>Mn*
4.7	*Mg>Fe>Ca>Cu>Mn*	*Mg>Fe>Ca>Cu>Mn*
5.7	*Fe>Mg>Ca>Cu>Mn*	*Fe>Mg>Ca>Cu>Mn*
6.8	*Fe>Mg>Ca>Cu>Mn*	*Fe>Mg>Ca>Cu>Mn*
8.6	*Mg>Fe>Ca>Cu>Mn*	*Mg>Fe>Ca>Cu>Mn*
10.8	*Mg>Fe>Ca>Cu>Mn*	*Mg>Ca>Fe>Cu>Mn*

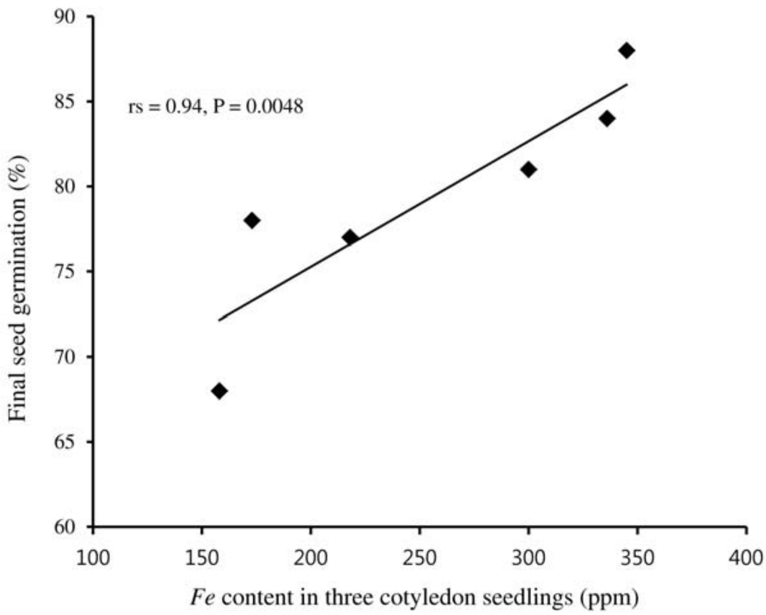

Fig.2. Correlation between *Fe* content in three cotyledon seedlings and percentage of final seed germination of *P. harmala*.

formation of multiple cotyledons. The expression of several genes encoding late embryogenesis abundant proteins is included in *Fe*-deficiency roots and/or leaves (Kobayashi *et al*., 2009).

Soil salinity in the oasis Ekhiin Gol could affect for embryogenesis, seed germination and seedling nutrient absorption of *P. harmala*. This species probably will become extinct in this oasis, if soil salinity increases under natural and anthropogenic effects (Pankova *et al*., 2004). The results indicate the variation in cotyledon number of *P. harmala* is related with soil salinity, resulting in at least *Fe* deficiency.

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