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Effect of irrigation with saline water on ion homeostasis and forage dry yield in Alfalfa ecotypes application of high salty water for Alfalfa plants irrigation

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Abstract

Salinity stress is a brutal environmental stress which decreases the yield production of plants. Questions rise on which of the ionic stress or lack of water has deleterious effects on plants forage dry yield. Also, questions remain on whether the K^+ reduction or Na^+ accumulation is more important in forage dry yield reduction under salinity stress. The present experiment was conducted to answer the above questions in four alfalfa ecotypes. To do so, 6-7 weeks seedlings were irrigated with high salty water (EC=20dS m^{-1}) and RWC, MSI, height, forage dry yield, Na^+ and K^+ were measured 1, 3, 6, 10, and 16 days after the salt shock. The results showed that one day after irrigation with saline water, all measured traits changed adversely. Salinity stress by decreasing K^+ and increasing Na^+ content reduced the growth of alfalfa plants. RWC reduction was less than K^+ reduction or Na^+ accumulation, so ionic stress had more deleterious effects on forage dry yield of alfalfa plants. Root cells had a higher content of K^+ and Na^+ ions compared with leaves, hence, they had a major defensive role against salinity stress. The K^+/Na^+ ratio reduction in saline condition was the main element for decreasing plant forage dry yield. The application of high salty water for irrigation of alfalfa plants is possible if there is a good subsoil drainage system to remove the leached saline water regularly from the soil. It is also suggested that foliar application of potassium may be ameliorate harmful effects of salinity stress in plant growth.

Keywords: Potassium; Sodium; Irrigation with Saline Water; K^+/Na^+ Ratio; Alfalfa

1. Introduction

Soil salinity is a harsh environmental stress for crop production that has affected more than 45 million hectares of irrigated lands. It has increased with global climate changes and incorrect agricultural practices (Munns and Tester, 2008; Roy *et al.*, 2014). Salinity in soil and/or irrigation waters leads to significant reduction of agricultural production (Maas and Hoffman, 1977). Salinity stress inhibits plants seed germination and growth by affecting gas exchanges, photosynthesis, water relation, protein synthesis, energy production and the whole growth process (Parida and Das, 2005). The main adverse effects of salinity on plant growth and development consist of (i) water stress, (ii) ionic stress, (iii) nutritional imbalance (Ashraf and Foolad, 2013). Plants use various physiological and biochemical mechanisms for their survival under salt stress conditions such as (i) ion transportation, (ii) ion homeostasis, (iii) biosynthesis of compatible solutes (Ashraf and Akram, 2009). In low salinity stress, osmotic pressure is the main reason behind decreasing growth and forage dry yield. However, in high salinity, ionic stress does the same work (Munns and Tester, 2008). In saline condition, the build-up of saline ions in leaves leads to toxicity damage in the later stages of growth and can be

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reduced by limiting Na^+ transfer to the shoot tissue and compartmentalization of Na^+ into the root tissue (Testerink, 2014). Ion regulation or homeostasis is an important trait under normal and saline conditions for plant growth and yield production (Aşık *et al.,* 2009). Salinity stress disturbs the nutritional balance and results in higher levels of Na^+/K^+, so leads to plant growth retardation (Grattan and Grieve, 1999).

Alfalfa (Medicago sativa) is one of the most extensively cultivated perennial forages in the world. It has nitrogen-fixing capabilities, high yield and quality and agronomic importance (Babakhani *et al.,* 2011). Alfalfa is moderately salt-tolerant as compared with other legumes. Alfalfa production starts to decrease at salinity above a 2 dS m^{-1} and shows a 7.3% reduction for each dS m^{-1} (Maas and Hoffman, 1977). In many parts of Iran, perceptions occur in fall, winter and early spring, but the agricultural season starts from late spring to the end of summer. Water shortage in summer is obviously sensible in the West and the North-West of Iran so crop production in the hot and dry months of summer is possible only with water supply or irrigation. Moreover, thirteen permanent rivers flow into Urmia Lake, where most of them contain high salinity ions (Eimanifar and Mohebbi, 2007). Previous studies (Farahbakhsh and Toufighi, 1997) have shown that the salty content of saline soils in Iran predominately contains cations (Na^+, Ca^{++} (90%)) and anions (Cl^- 50% in normal soils and 80% in saline soils). Therefore, attention to the use of saline waters for irrigation of plants increases by the day due to water scarcity.

Hence, these questions arise:
(i) Is it the ion toxicity or the water stress that is more deleterious for forage dry yield?
(ii) Is it K^+ reduction or Na^+ accumulation that is more important in forage dry yield reduction under salinity stress?
(iii) Is it the roots or leaves that are affected seriously when encountered with saline ions?
(iv) How many days do ions take to accumulate in roots and leaves?
(v) How do ions affect plants growth?
(vi) How do changes in saline ions affect plants growth?

2. Materials and Methods

2.1. Germination Test

Four alfalfa ecotypes were obtained from the Iranian seed and plant improvement institute (http://www.spii.ir). The experiment design was factorial and comprised of four levels of salt (0, 5, 10, and 15 dS m^{-1}) with four alfalfa ecotypes (Tasuj, Local253, France and Zarrinshahr) in completely randomized design. Seeds of ecotypes were scarified for two minutes in 98% sulphuric acid and then surface-sterilized for three minutes in 5% sodium hypochloride. After washing, 50 seeds of each ecotype were subjected to standard germination test in Petri dishes with three replications. Petri dishes were put in an incubator at 23°C and in the dark and germination indices were recorded every two days until eight days. Radicle length, plumule length and seedling fresh weight were measured at the eighth day by ruler (cm) and laboratory balances, respectively. The following germination indices were calculated:
(i) Final germination percentage; seeds germinated/total seeds × 100
(ii) Seed vigor: germination percentage × plumule length
(iii) Germination index: $(8\times n_2)+(6\times n_3)+(4\times n_4)+(2\times n_5)$, where n=number of seeds newly germinated at time (Al-Mudaris 1998).

2.2. Plants Material, Growth Condition and Experimental Design:

The seeds of the above-mentioned ecotypes were cultivated in three lines with 14 cm distance in fiberglass tanks after treatment with thiram fungicide. The fiberglass tanks (1×2×0.6 m) were filled with sand to 10cm of their height and then were filled with farm soil (Table 1). Also, there was a fine drainage in the bottom of each fiberglass tank, which could remove water. The treatments were irrigated with tap water until 6-7 weeks after seedling establishment. Salt shock was imposed by irrigation with high volume of saline water (EC=20 dS m^{-1}) twice a week. Irrigation with saline water caused to increase the soil EC from 1.4 (before salt shock) to 10.8 dS m^{-1} (after 16 days). The study considered that there was no water shortage around the plants roots, so plants were always well watered. Saline water was prepared according to Leland *et al*., (1994); NaCl and $CaCl_2$ concentration in the water solution were equal (1:1). The experiment design was factorial and comprised of two levels (control and salt shock) with four alfalfa ecotypes and three replications. Root and leaf samples were taken to evaluate biochemical traits on 1, 3, 6, 10 and 16 days after the salt shock.

Table 1. Soil properties for agricultural experimental station of University of Zanjan

Soil texture	Loam	Bulk density	1.59
EC (dS m^{-1}) before irrigating with saline water	1.4	Organic matter	1.11
EC (dS m^{-1}) after irrigating with saline water	10.8	pH	7.60

2.3. Measurements

2.3.1. Forage Dry Yield

Forage dry yield was measured by cutting the whole plants after 10% of the beginning of flowering and at the end of experiment, and dry yield expressed as gram per plant after drying it in the oven at 55ºC for 72 hour (Neres *et al.*, 2010).

2.3.2. Relative Water Contents (RWC)

Fresh leaves were detached from each treatment and replication and weighed immediately to measure fresh weight (FW). After that, the samples were dipped in distilled water for 12 h. The leaves were weighed to record fully turgid weight (TW) and were subjected to oven drying at 70˚C for 24 h to record the dry weight (DW). The RWC were calculated by the equation of RWC= [FW-DW] / [TW-DW] ×100 (Smart and Bingham, 1974).

2.3.3. Membrane Stability Index (MSI)

MSI estimated by the method of Sairam *et al.,* (1997). Fresh leaf sample (0.1 g) was taken in 10 cm^3 of double distilled water in two sets. One set was subjected to 40°C for 30 min and its conductivity was recorded using a conductivity meter (C1). The second set was kept in a boiling water bath (100°C) for 10 min and its conductivity was also recorded (C2). Membrane stability index (MSI) = [1 - (C1/C2)] × 100.

2.3.4. Ions Content

A hundred milligram ground of the dry mass of roots and leaves was put in 10ml of distilled water at 25˚C for 1h. The homogenates were centrifuged at 3000×*g* for 15 min and the supernatants were filtered through qualitative filter paper (Da Silva *et al*., 2008). The extracts were used to determine sodium and potassium content. To determine Na^+ and K^+ content by flame photometry, an aliquot of filtrate was used (Sarruge and Haag, 1974).

2.3.5. Statistical Analysis

The Data were subjected to analysis of variance (ANOVA) and the means were compared by using Duncan's range test at $P < 0.05$. All calculations were performed using SAS software, version 9.4.

3. Results

3.1. Germination Evaluation Indices

Salinity stress decreased germination percentage, seed vigor, germination index, radicle length, plumule length and fresh weight of alfalfa ecotypes seedling compared to the no saline treatment. Germination index reduction was more severe by increasing salinity concentration (Table 2). There was no important difference between germination indices among the ecotypes at low salinity concentration (5dS m^{-1}) but Tasuj and Local253 ecotypes had significantly higher germination indices compared with Zarrinshahr and France ecotypes at above 10dS m^{-1} salinity concentrations.

3.2. Relative Water Content (RWC) and Membrane Stability Index (MSI):

The analysis of variance showed the effects of irrigation with saline water (S), ecotypes (E), the time of sample taking and E×T interaction on RWC were significant (Table 3). RWC decreased sharply in all ecotypes a day after irrigation with saline water, but improved slowly after three days in comparison with the first day, however; it was significantly lower than the control. Tasuj and France ecotypes had the highest and the least RWC a day after the salt shock compared with other ecotypes, respectively. However, after three days, Tasuj had higher RWC compared with other ecotypes (Table 4). RWC decreased in France, Local253, Tasuj and Zarrinshahr 27%, 28%, 20% and 26%, respectively after 16 days of salt shock. The analysis of variance showed the effects of irrigation with saline water (S), the time of sample taking and S×T interaction on MSI were significant (Table 3). MSI didn't change significantly in all ecotypes a day after irrigation with saline water but decreased after three days. MSI reduction continued by the day until 16 days, and there was no significant difference among ecotypes until 10 days after irrigation with saline water. Zarrinshahr had the least MSI among the ecotypes, and there was no significant difference among other ecotypes 16 days after irrigation with saline water (Table2).

Table 2. The means of Germination indices at (0, 5, 10, and 15ds/m) NaCl concentrations in 4 alfalfa ecotypes; GP: germination percent; GI: germination index; RL: Radicle length; PL: Plumule length; SV: Seed Vigor, SFW: seedling Fresh weight

Salt levels	Ecotypes	GI	GP	GR	PL	SV	SFW (mg)
	France	4.16	86.67	1.81	2.42	210.00	0.14
Control	Local253	4.64	90.67	1.90	2.76	252.33	0.10
	Tesoj	4.20	88.00	1.96	2.97	262.00	0.14
	Zarrinshahr	5.17	97.33	3.28	3.21	312.00	0.14
	France	3.40	69.33	1.40	1.89	131.33	0.11
Salinity Stress	Local253	3.71	80.00	1.22	1.95	157.33	0.15
5 dS m^{-1}	Tesoj	3.96	85.33	1.76	2.09	179.33	0.17
	Zarrinshahr	2.91	61.33	1.25	1.35	83.00	0.07
	France	1.65	41.33	0.13	0.43	17.67	0.06
Salinity Stress	Local253	2.84	72.00	1.14	1.83	132.67	0.11
10 dS m^{-1}	Tesoj	3.12	72.00	1.81	1.91	137.00	0.07
	Zarrinshahr	1.75	37.33	0.35	1.01	37.67	0.05
	France	0.32	9.33	0.07	0.00	0.00	0.00
Salinity Stress	Local253	0.80	25.33	0.58	0.03	0.86	0.02
15 dS m^{-1}	Tesoj	0.97	34.67	0.69	0.04	1.21	0.03
	Zarrinshahr	0.35	16.00	0.16	0.00	0.00	0.01
LSD (α=0.05)		0.32	6.70	0.30	0.24	25.00	0.02

Table 3. Analysis of variance of relative water content (RWC), membrane stability index (MSI), sodium (Na^+), potassium (K^+), and K^+/Na^+ ratio, Na^+ root / Na^+ leaf ratio (RNa^+ / LNa^+, and K^+ root / K^+ leaf ratio (RK^+ / LK^+) under salinity stress(20dS m^{-1}) in 4 alfalfa ecotypes after (1, 3, 6, 10 and 16) days

Source	Freedom degree	RWC	MSI	Na^+ Leaf	K^+ Leaf	K^+/Na^+ Leaf	Na^+ Root	K^+ Root	K^+/Na^+ Root	RNa^+ / LNa^+	RK^+/ LK^+
Block	2	4.72ns	3.30ns	4.45*	10.30*	0.13ns	0.89ns	5.76ns	0.02ns	0.18*	0.08ns
Salinity (S)	1	16291.83*	5258.00*	1449.01*	499.68*	253.46*	2245.40*	720.30*	161.70*	0.22*	1.14*
Ecotype (E)	3	94.22*	3.34ns	14.67*	110.53*	1.16*	158.20*	110.46*	6.47*	2.22*	1.14*
S × E	3	23.38ns	2.40ns	1.42*	6.83*	0.28ns	0.69ns	1.58ns	0.04ns	0.02ns	0.05ns
Error a	10	32.84*	392.70*	29.64*	304.94*	3.86*	31.10*	10.30ns	0.21ns	0.04ns	1.7*
Time (T)	4	52.35*	544.30*	33.58*	463.13*	9.68*	28.46*	53.99*	1.35*	0.11*	1.79*
S × T	4	9.29ns	14.20*	2.44*	23.51*	0.52*	1.20*	5.73ns	0.17*	0.04*	0.09*
E × T	12	62.28*	11.50ns	14.89*	6.27*	2.20*	133.1*	91.2*	0.63*	1.38*	0.1*
E × S ×T	12	4.74ns	8.02ns	2.35*	14.38*	0.69*	1.08*	7.59ns	0.23*	0.05*	0.17*
Error	70	8.12	5.30	0.26	1.76	0.15	0.52	4.04	0.08	0.02	0.03
CV		3.69	12.7	6.09	6.44	12.05	6.27	8.17	10.83	9.56	13.32

3.3. Forage Dry Yield

The analysis of variance showed that the effects of irrigation with saline water (S), ecotypes (E), and E×T interaction on forage dry yield were significant (Table 5). Irrigation with saline water decreased the plant height and forage dry yield in all ecotypes significantly 16 days after irrigation with saline water (Figure 1). Salinity decreased forage dry yield in Tasuj, Local253, France and Zarrinshahr ecotypes 24%, 32%, 33% and 33% compared to the control, respectively. Tasuj had the highest forage dry yield and height in saline and control conditions and its growth in saline condition decreased less than other ecotypes. There was no significant difference among Zarrinshahr, France, Local253 ecotypes in forage dry yield in saline condition (Figure 1).

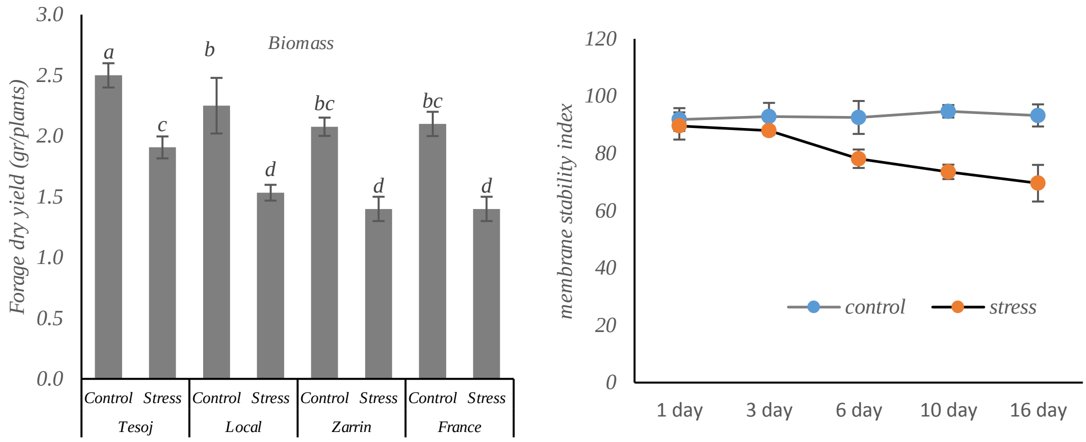

Fig. 1. Mean value of Forage dry yield (after 10% starting flowering) and membrane stability index (MSI) in leaves of four alfalfa ecotypes during 16 days after irrigation with saline water

Table 4. Mean value for relative water content (RWC), memberane stability index (MSI), sodium (Na^+), potassium (K^+), K^+/Na^+ ratio, Na^+ root / Na^+ leaf ratio (RNa^+/LNa^+) and K^+ root / K^+ leaf ratio (RK^+/LK^+) in four alfalfa ecotypes after irrigating with saline water (EC=20 dS m^{-1})

Time	Ecotypes	Treatments	RWC	MSI	Na^+ Leaf (mmol gr)	K^+ Leaf (mmol gr)	K^+/Na^+ Leaf	Na^+ Root (mmol gr)	K^+ Root (mmol gr)	K^+/Na^+ Root	RK^+/LK^+	RNa^+/LNa^+
after 1 day	France	Control	89	90	5.6	18.7	3.3	5.7	26.0	3.9	1.40	1.19
		Salt shock	65	87.2	8.2	25.1	3.1	10.5	22.3	2.1	0.89	1.29
	Local253	Control	90	90.5	4.9	21.3	4.4	5.7	29.0	4.3	1.36	1.38
		Salt shock	65	87.4	10.1	27.7	2.8	11.6	23.3	2.0	0.84	1.16
	Tesoj	Control	88	90.3	4.7	26.2	5.6	6.0	21.7	3.1	0.82	1.52
		Salt shock	70	88.4	11.1	35.7	3.2	12.1	26.0	2.2	0.73	1.10
	Zarrinshahr	Control	91	92.5	5.0	17.7	3.6	6.1	21.3	3.0	1.20	1.43
		Salt shock	67	88.3	8.4	28.3	3.4	18.7	24.3	1.3	0.86	2.24
After 3 days	France	Control	84	90.5	5	19.3	3.9	5.7	32.0	4.8	1.67	1.36
		Salt shock	66	85.4	8.5	22.1	2.7	11.4	23.0	2.1	1.06	1.36
	Local253	Control	90	93.3	4.7	21.6	4.6	5.8	29.7	4.4	1.38	1.44
		Salt shock	68	86.9	10.3	19.1	1.9	12.1	21.7	1.8	1.13	1.17
	Tesoj	Control	91	94	5.3	28.3	5.3	6.8	24.3	3.2	0.86	1.46
		Salt shock	69	85.9	14.4	23.7	1.6	13.1	24.3	1.9	1.03	0.92
	Zarrinshahr	Control	88	93.6	5.0	22.8	4.6	6.9	22.7	2.9	1.00	1.60
		Salt shock	70	86.6	9.5	25.1	3.7	19.9	24.0	1.2	0.81	2.49
After 6 days	France	Control	91	92.1	4.7	20.6	4.5	5.9	30.0	4.4	1.48	1.47
		Salt shock	67	75	10.7	16.0	1.5	12.8	24.7	1.9	1.54	1.20
	Local253	Control	89	91.3	4.9	23.3	4.8	6.3	29.7	4.1	1.27	1.48
		Salt shock	67	77.3	10.8	18.8	1.7	13.2	20.0	1.5	1.08	1.22
	Tesoj	Control	89	93.1	4.6	23.4	5.1	6.1	27.3	3.8	1.17	1.55
		Salt shock	75	80.5	14	18.3	1.3	14.0	21.7	1.5	1.19	1.01
	Zarrinshahr	Control	89	93.8	4.4	21.6	4.9	6.2	23.0	3.2	1.07	1.65
		Salt shock	69	79.8	11.6	13.8	1.2	23.3	22.0	0.9	1.60	2.02
After 10 days	France	Control	88	94.8	5.3	22.5	4.3	6.5	31.7	4.2	1.42	1.43
		Salt shock	67	74.5	13.3	12.3	0.9	14.3	24.0	1.7	2.00	1.07
	Local253	Control	90	96	5.2	23.6	4.6	6.4	30.0	4.1	1.27	1.44
		Salt shock	69	72.8	11.1	15.3	1.4	14.0	23.0	1.6	1.51	1.26
	Tesoj	Control	88	93.7	5.1	25.0	4.9	5.9	27.7	4.0	1.11	1.36
		Salt shock	72	77.9	13.6	15.3	1.1	15.3	20.7	1.4	1.38	1.13
	Zarrinshahr	Control	89	91.4	4.6	22.5	4.9	6.2	23.3	3.3	1.04	1.55
		Salt shock	68	69.1	12.0	11.7	1.0	23.8	21.0	0.9	1.82	1.98
After 16 days	France	Control	85	93.9	4.9	22.7	4.7	6.0	31.0	4.4	1.37	1.47
		Salt shock	60	70.8	16.0	8.3	0.5	18.3	22.3	1.3	2.71	1.08
	Local253	Control	90	91.4	4.6	24.5	5.3	6.3	30.3	4.2	1.24	1.58
		Salt shock	62	70.7	13.7	7.3	0.5	16.8	18.0	1.1	2.50	1.16
	Tesoj	Control	91	90.9	4.7	23.7	5.1	5.5	28.0	4.4	1.18	1.38
		Salt shock	68	69.8	16.8	13.0	0.8	17.3	19.0	1.2	1.47	0.97
	Zarrinshahr	Control	90	90.8	5.1	23.0	4.6	6.7	24.7	3.0	0.98	1.55
		Salt shock	62	66.1	14.9	8.9	0.6	26.1	18.0	0.7	2.04	1.74
LSD (α=0.05)			5	3.2	1.5	2.1	0.7	1.5	2.9	0.5	0.18	0.16

Table 5. Analysis of variance of forage dry yield under salinity stress (20dS m-1) in 4 alfalfa ecotypes after 10% starting flowering

Source	Freedom degree	Forage dry yield
Block	2	0.0003ns
Salinity (S)	1	2.70*
Ecotype (E)	3	0.28*
S × E	3	0.004ns
Error	14	0.015
CV		6.53

3.4. Sodium Content in Leaves and Roots

The analysis of variance showed that the effects of irrigation with saline water (S), time of sample taking (T), ecotypes (E) and the S×T, E×T, E×T×S interactions on Na^+content in leaves and roots plus S×E interactions in leaves were significant (Table 3). Na^+ content in leaves and roots of all ecotypes increased significantly a day after irrigation with saline water (Figure 2, 3). Na^+ content in leaves and roots of all ecotypes increased by the day until 16 days after irrigation with saline water (Figure 2, 3). Na^+ content in leaves of Tasuj and Local253 increased more than other ecotypes a day after irrigation with saline water. Tasuj had the highest Na^+ content in its leaves after three days until 16 days after irrigation with saline water. Tasuj and Local253 ecotypes had the highest and the least sodium content in their leaves, respectively compared with other ecotypes 16 days after irrigation with saline water (Table 4). Sodium content in leaves of France, Local253, Tasuj and Zarrinshahr was 226%, 197%, 257% and 192%, respectively higher than control on 16 days after irrigation with saline water. Zarrinshahr had the highest sodium content in its root tissues compared with other ecotypes in the whole of the experiment after irrigation with saline water (Table 4). Sodium content in roots of France, Local253, Tasuj and Zarrinshahr increased 205%, 167%, 215% and 289%, respectively compared with their control 16 days after irrigation with saline water (Table 4).

3.5. Potassium Content in Leaves and Roots

The analysis of variance showed that the effects of irrigation with saline water (S), ecotypes (E), time of sample taking (T), and the E×T interactions on potassium content in leaves and roots plus S×E, S×T, E×T×S interactions in leaves were significant (Table 3). The curvy changes of K^+ content in roots and leaves of all ecotypes was descending during 16 days after irrigation with saline water (Figures 2, 3). However, K^+ content in the leaves of all ecotypes increased a day after irrigation with saline water, but after 3 days started to reduce (Figure 2). K^+ content in the leaves of all ecotypes was less significant than control six days after irrigation with saline water (Table 4). Investigation of the interactions showed that Tasuj had the highest K^+ content a day after irrigation with saline water compared with other ecotypes. Zarrinshahr and Tasuj ecotypes on the third day, Tasuj and Local253 on sixth and tenth day, Tasuj on sixteenth days had the highest K^+ content among the ecotypes. K^+ content reduction in leaves of France, Local253, Tasuj and Zarrinshahr was 63%, 70%, 45% and 62%, respectively 16 days after irrigation with saline water. Contrary to leaves, K^+ content in roots of Tasuj and Zarrinshahr ecotypes increased a day after the salt shock, but it decreased in France and Local253 (Table 4). K^+ content in roots of all ecotypes had no significant difference with the control on the third day, but it was significantly lower than the control on the sixth day. Tasuj on the first day, Tasuj and Zarrinshahr on the third day, France on sixth day, Local253 and France on the tenth and France on sixteenth day had the highest K^+ content among the ecotypes (Table 3). K^+ content in roots of France, Local253, Tasuj, and Zarrinshahr decreased 28%, 40%, 32% and 20%, respectively 16 days after irrigation with saline water (Table 4).

3.6. K^+/Na^+ Ratio Content in Leaves and Roots

The analysis of variance showed that the effects of irrigation with saline water (S), time of sample taking (T), ecotypes (E) and the S×E, S×T, E×T, E×T×S interactions on K^+/Na^+ ratio in leaves and roots were significant (Table 3). K^+/Na^+ ratio in leaves of Local253 and Tasuj ecotypes decreased significantly a day after irrigation with saline water, but it had no significant change in France and Zarrinshahr (Table 4). Zarrinshahr had the highest K^+/ Na^+ ratio compared with other ecotypes on the third day after the salt shock, but in later days there were no significant differences among ecotypes in K^+/ Na^+ ratio of leaves. K^+/ Na^+ ratio in roots of all ecotypes decreased sharply a day after irrigation with saline water and its reduction continued in all ecotypes until the end of the experiment (Figures 1, 2). Zarrinshahr had the least K^+/Na^+ ratio in its roots compared with other ecotypes, and there were no significant differences among other ecotypes during 16 days after irrigation with saline water (Table 3).

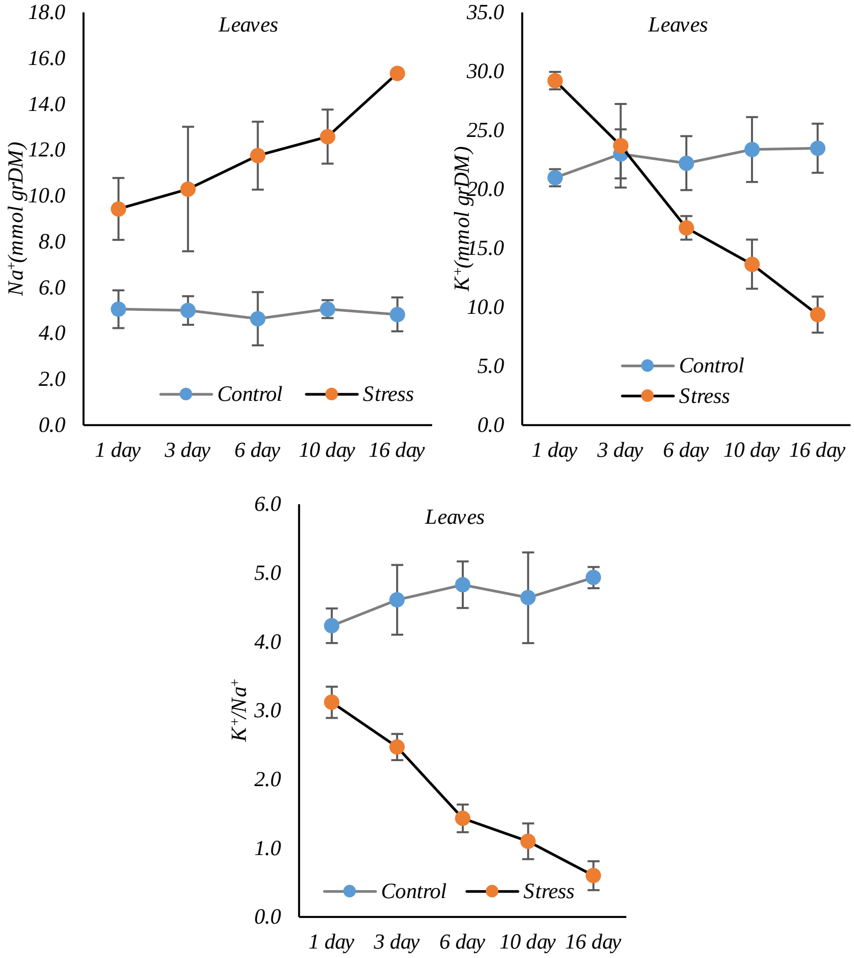

Fig. 2. Mean value of sodium (Na^+), potassium (K^+), and K^+ / Na^+ ratio changes in leaves of four alfalfa ecotypes during 16 days after irrigation with saline water

3.7. Na^+ Roots/Na^+ Leaves Ratio (NR/NL)

The analysis of variance showed that the effects of irrigation with saline water (S), ecotypes (E) and the S×T, E×T, E×T×S interactions on NR/NL were significant (Table 3). NR/NL increased in Zarrinshahr and France ecotypes a day after irrigation with saline water, but decreased in other ecotypes (Table 4). NR/NL in all ecotypes was lower than the control during 16 days in saline condition except Zarrinshahr. NR/NL in Zarrinshahr ecotype at salinity condition was higher than control, and also Zarrinshahr had the highest NR/NL ratio among the ecotypes (Table 4).

3.8. K^+ Roots/K^+ Leaves Ratio (KR/KL)

The analysis of variance showed that the effects of irrigation with saline water (S), time of take a sample (T), ecotypes (E) and the S×T, E×T, E×T×S interactions on KR/KL were significant (Table 3). The KR/KL ratio decreased significantly in all ecotypes a day after the salt shock, but increased after 10 days in all ecotypes except Tasuj (Table 4). Tasuj on the third day, Zarrinshahr on the sixth day, and all ecotypes after 10 days had higher KR/KL ratio compared with the control (Table 4). France ecotype had the highest and Tasuj ecotype had the lowest KR/KL ratio among the ecotypes 16 days after salt shock (Table 4). Tasuj had the least changes in KR/KL ratio among other ecotypes during 16 days after irrigation with saline water (Table 4).

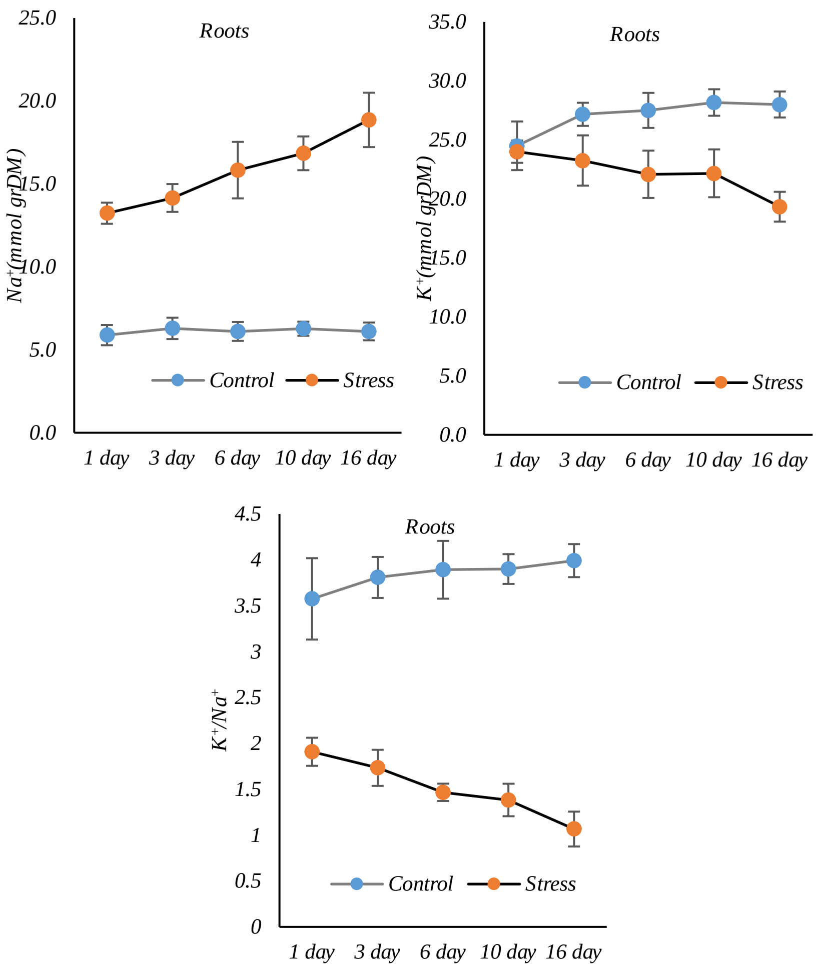

Fig.3. Mean value of sodium (Na^+), potassium (K^+), and K^+ / Na^+ ratio in roots of four alfalfa ecotypes during 16 days after irrigation with saline water

4. *Discussion*

4.1. Germination Indices Under Salinity Stress

Salinity stress decreased germination indices in all ecotypes because of osmotic potential reduction or the toxic effects of Na^+ and Cl^- ions (Khajeh-Hosseini *et al.*, 2003). Germination index reduction in alfalfa plants was reported by Wang *et al*., (2009), Li *et al*., (2010), Cornacchione and Suarez (2015), and Ansari *et al.*, (2017). Salinity stress in low concentration causes to induce dormancy in seeds and decrease the germination rate, however, it kills the seeds and decreases germination percentage in many plants at high concentration (Shannon and Grieve, 1999; Ibrahim, 2016). According to the results of the germination test at different salinity concentration, Tasuj and Local253 ecotypes had a higher germination performance, so they were selected as saline tolerant ecotypes in germination stage. Also, Zarrinshahr and France ecotypes had a weak germination performance, so they were selected as sensitive ecotypes in the germination stage. However; germinability under saline condition is not related to salinity tolerant at other stages (Al-Niemi *et al.*, 1992). This selection was done to investigate the effects of irrigation with saline water on growth and forage dry yield, in both saline tolerant and saline sensitive ecotypes in germination stage.

4.2. RWC and MSI

Decreasing RWC and MSI in alfalfa plants under salinity stress were reported by Wang *et al.*, (2009); Mhadhbi *et al.*, (2013); Campanelli *et al.*, (2013). The most biochemical and physiological processes in cells such as cell dividing, cell enlargement, stomata opening, photosynthesis rate, mineral and assimilates transportation depend on water status (Munns 2002; Parida and Das, 2005; Gupta and Huang, 2014), so the reduction in water content could decrease plants growth and yield. Tasuj, saline tolerant in the germination stage, had a high RWC, which means that it had a high ability for water up-taking from the saline soil. In this study, RWC in saline condition decreased sharply a day after irrigation with saline water and after that had the least changes until 16 days later (Table 4). RWC was lower than the control, but it improved slowly three days after irrigation with saline water. So, it seems that water shortage is not the main issue responsible for plant death or forage dry yield reduction in saline condition. Cell Membrane Index is one of the important markers of oxidative stress in plant cells (Gill and Tuteja, 2010). Salinity stress causes reactive oxygen species (ROS) content in plant cells to increase. ROS attack macromolecules such as proteins, DNA, enzymes and membrane lipids and cause injury to the membranes of cells under salinity stress and disturb the nutrient balance (Kaya *et al.,* 2009; Gill and Tuteja, 2010). Some researchers reported that MSI reduction in saline stress is related to the accumulation of salt ions and the replacement of K^+ sitting bound by Na^+ (Parida and Das, 2005; Gill and Tuteja, 2010). Also, it is reported that the overproduction of ROS in salinity condition leads to damage membrane stability and induces K^+ leak from the cell (Cuin and Shabala, 2007).

4.3. Height and Forage Dry Yield

Height and forage dry yield reduction in alfalfa ecotypes in saline condition are reported by Cornacchione and Suarez (2015) and Emam *et al*., (2009). The primary effect of salt stress on plant growth is the reduction of water uptake by salinity osmotic effect (Levitt, 1980). The accumulation of toxic ions in leaves causes to induce senescence and the elder leaves to fall. It also reduces the photosynthesis rate. Reducing the photosynthesis rate decreases the transfer of assimilates to growing tissue and the growth of the whole plant (Munns 2002). It is predicted that forage dry yield reduction should be more than 131% at 20 dS m^{-1} (Maas and Hoffman, 1977). In this study, maximum reduction under saline stress was 33% (Figure 1), so the application of high salty water to irrigate alfalfa plant is possible if there is a good subsoil drainage system to ensure that leached saline water can regularly be removed from the soil.

4.4. Ions Content

Significant reduction of K^+ and K^+/Na^+ ratio in salinity stress was indicated by accumulating Na^+ ions in alfalfa plant (Table 4). Similar results in alfalfa plant was reported by Cornacchione and Suarez (2015); Campanelli *et al.,* (2013); Emam *et al.,* (2009).

4.5. Sodium

Na^+ ion is one of the most important and predominant ions in salinity stress that is assumed to be the main factor responsible for the reduction of growth and forage dry yield under salinity (Hong *et al.,* 2009). Na^+ ion interferes with K^+ and Ca^{2+} ions, closes the stomatal, and decreases photosynthesis, growth and forage dry yield (Tavakkoli *et al.,* 2010). Tasuj, with high Na^+ content in its leaves had a high forage dry yield compared with other ecotypes (Table 4). It seems that Tasuj, by compartmenting Na^+ ions into leaf cells vacuoles can decrease the toxic effects of Na^+. Na^+ sequestration and compartmentation into vacuole is one of the important mechanisms against salinity stress (He *et al.,* 2015). Na^+ sequestration also causes to improve the cellular osmotic potential and salinity tolerance (Szabados and Savoure, 2010). Zarrinshahr with high Na^+ content in its roots could not decrease the toxic effects of Na^+ on its growth. In fact, Zarrinshahr tried to minimize salt ions accumulation in its leaf tissue to survive and produce forage dry yield. But it seems that compartmentalization of Na^+ ions into leaf cells vacuole was more effective than roots for decreasing harmful effects of salinity stress. Compared with leaves, accumulation of high Na^+ in roots at the first day showed that all ecotypes at first tried to inhibit from entering Na^+ ions to their shoots and leaves by compartmenting ions into roots. But after several days due to uncontrollable entering of saline ions into roots, they could not control entering Na^+ ions into their shoots and leaves (Figure 1). Potassium: K^+ is one of the most abundant cations in the plant cell, highly mobile, distributed between vacuole and cytoplasm and is required for photosynthesis and proteins synthesis (Collins, 2012). Potassium had a critical role in salinity tolerance and in adjusting osmotic pressure (Maathuis and Amtmann 1999). In the present study, K^+ content in leaves of all ecotypes increased a day after salt shock (Figure 1). It seems that plant cells, by increasing the absorption of available K^+ from root zone, tried to adjust their osmotic potential in saline condition (Wang *et al.,* 2013). By increasing the time of irrigation with saline water stress, K^+ content obviously decreased in all ecotypes. Tasuj and France ecotypes had the highest K^+ content in leaves and roots, respectively after 16 days of salinity shock. But only Tasuj ecotype had the highest forage dry yield in saline condition. The results also showed that K^+ content in root tissues of all ecotypes was more than leaf tissues (Table 4). Potassium in leaves in addition to adjusting osmotic potential is used in the photosynthesis process (Wang *et al.*, 2013). It appears that high K^+ content in leaves is more effective than K^+ content in roots for producing forage dry yield in saline condition. Hence, it is suggested that foliar application of potassium can ameliorate harmful effects of salinity stress in plant growth.

4.6. K^+/Na^+ Ratio

Cytoplasmic K^+/Na^+ is an effective indicator of salinity tolerance (Zheng *et al.*, 2008) due to maintaining enzymes functions and plant growth in the saline condition. K^+/Na^+ reduction in saline condition is related to a reduction in K^+ content and an increase in Na^+ content in plants tissues (Poustini *et al.*, 2007) or direct competition between K^+ and Na^+ ions in up taking sites or the effect of Na^+ on K^+ transfer into the xylem, or a Na^+-increased K^+ efflux from the root (He and Cramer, 1993; Shabala *et al*., 2003). In this study also, K^+/Na^+ in salt stress decreased obviously in all ecotypes because of excessive Na^+ accumulation in the cytosol and K^+ content decrease in the cell (Table 4). Also, Na^+ content rate increase was more than K^+ content decrease under salinity condition in all ecotypes (Table 4). Tasuj ecotype, one of the saline tolerant ecotypes at germination stage, had a higher K^+/Na^+ compared with other ecotypes in saline condition (Table 2, 4). K^+/Na^+ in the roots of all ecotypes was more than the leaves significantly in the end of experiment (Table 4). K^+ content in the roots of all ecotypes was more than K^+ content in leaves, however, Na^+ content in roots was more than in leaves (Table 4). Therefore, it appears that all ecotypes, tried to maintain the up-taking of K^+ ions and inhibit entering K^+ ions by xylem transport system in saline condition. NR/NL and KR/KL: Na^+ content in the roots of France and Zarrinshahr was higher than the leaves, which shows that the roots firstly inhibit Na^+ transfer into to the leaves. However, in Tasuj and Local253 ecotypes, Na^+ could transfer within the whole plant (Table 4). KR/KL reduction in all ecotypes, a day after the salt shock, supported the available K^+ transportation from roots to leaves. By increasing the time of salinity shock (after three days), transporting potassium from roots to leaves decreased in all ecotypes until 16 days (Table 4). The main reason for reducing potassium content in saline condition was related to decreasing K^+ discrimination from sodium by plant roots (Maathuis and Amtmann 1999). Also, increasing KR/KL in all ecotypes 3 days after the salt shock showed that the root cells need more potassium compared with leaves to adjust their ions homeostasis and osmotic potential. Tasuj and Local253 ecotypes were saline tolerant at germination stage, but only Tasuj had a high RWC, Na^+, K^+ and K^+/Na^+ in saline condition compared with other ecotypes, therefore, the high forage dry yield of Tasuj under saline condition was related to above traits.

5. Conclusion

Salt shock changed adversely measured-traits in all alfalfa ecotypes a day after irrigation with high salty water. RWC decreased sharply a day after the salt shock, but improved after three days compared with the first day, so alfalfa ecotypes could recover and adjust their osmotic potential in less than three days. K^+ ions increased drastically in leaves of all ecotypes a day after irrigation with saline water, but decreased after three days until 16 days. Alfalfa ecotypes immediately increased K^+ absorption from roots after salt shock and K^+ content increased in their leaves. Na^+ content in roots of all ecotypes increased more than in leaves. Roots of all ecotypes inhibited slowly, transferring sodium to leaves after 3days. Roots had a higher content of sodium in their cells compared with the leaves. Tasuj, with high forage dry yield in saline condition, had a higher RWC, height, forage dry yield, sodium, potassium and K^+/Na^+ ratio. The results of this study suggest that in saline condition, decreasing potassium content and great increasing sodium content caused to decrease the K^+/Na^+ ratio. So K^+/Na^+ ratio reduction is the main element for decreasing alfalfa plants forage dry yield under saline condition.

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References

Al-Mudaris, M.A., 1998. Notes on various parameters recording the speed of seed germination. JARTS, 99; 147-154.

Al-Niemi, T.S., W.F. Campbell, M.D. Rumbaugh, 1992. Response of alfalfa cultivars to salinity during germination and post-germination growth. Crop Science, 32; 976–980.

Ansari, M., F. Shekari, M.H. Mohammadi, B. Biró, G. Végvári, 2017. Improving germination indices of alfalfa cultivars under saline stress by inoculation

with beneficial bacteria. Seed Science and Technology, 45; 1-10.
Ashraf, M., M. Foolad, 2013. Crop breeding for salt tolerance in the era of molecular markers and marker-assisted selection. Plant Breeding, 132; 10–20.
Ashraf, M., N. A. Akram, 2009. Improving salinity tolerance of plants through conventional breeding and genetic engineering: an analytical comparison. Biotechnol Adv., 27; 744–752.
Aşık, B.B., M.A. Turan, H. Celik, A.V. Katkat, 2009. Effects of humic substances on plant growth and mineral nutrients uptake of wheat (*Triticum durum* cv. Salihli) under conditions of salinity. Asian J Crop Sci., 1; 87–95.
Babakhani, B., R.A. Khavari-Nejad, H.R. Sajedi, H. Fahimi, S. Saadatmand, 2011. Biochemical responses of Alfalfa (Medicago sativa L.) cultivars subjected to NaCl salinity stress. African Journal of Biotechnology, 10; 11433-11441.
Campanelli, A., C. Ruta, I. Morone-Fortunato, G. De Mastro, 2013. Alfalfa (Medicago sativa L.) clones tolerant to salt stress: in vitro selection. Central European Journal of Biology, 8; 765-776.
Collins, R.P., 2012. The role of calcium and potassium in salinity tolerance in brassica rapa L. cv. RCBr Seed (Doctoral dissertation, Coventry University).
Cornacchione, M.V., D.L. Suarez, 2015. Emergence, forage production, and ion relations of Alfalfa in response to saline waters. Crop science, 55; 444-457.
Cuin, T.A., S, Shabala, 2007. Compatible solutes reduce ROS induced potassium efflux in Arabidopsis roots. Plant Cell Environ., 30; 875–885.
Da Silva, E.C., R.J.M.C. Nogueira, F.P. De Araújo, N.F. De Melo, A.D. De Azevedo Neto, 2008. Physiological responses to salt stress in young umbu plants. Environmental and Experimental Botany, 63; 147-157.
Eimanifar, A., F. Mohebbi, 2007. Urmia Lake (northwest Iran): a brief review. Saline syst, 3; 1-8.
Emam, Y., E. Bijanzadeh, R. Naderi, M. Edalat, 2009. Effects of salt stress on Vegetative growth and ion accumulation of two alfalfa (Medicago sativa L.) cultivars. Desert, 14; 163-169.
Farahbakhsh, M., H. Toufighi, 1997. Relationships between electrical conductivity and total soluble salts content in salt affected soils of Iran. Iranian journal of agricultural science (in Persian with English abstract), 28; 86-96.
Gill, S., N. Tuteja, 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem, 48; 909-930.
Grattan, S.R., C.M. Grieve, 1999. Salinity–mineral nutrient relations in horticultural crops. Sci Hort., 78; 127–157.
Gupta, B., B. Huang, 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. International Journal of Genomics, 20; 1-18.
He, T., G.R. Cramer, 1993. Salt tolerance of rapid cycling *Brassica s*pecies in relation to potassium/sodium ratio and selectivity at the whole plant and callus level. Journal of Plant Nutrition, 16; 1263-1277.
He, Y., J. Fu, C. Yu, X. Wang, Q. Jiang, J. Hong, K. Lu, G. Xue, C. Yan, A. James, L. Xu, U. Xu, J. Chen, D. Jian, 2015. Increasing cyclic electron flow is related to Na^+ sequestration into vacuoles for salt tolerance in soybean. Journal of Experimental Botany, 66; 6877-6889.
Hong, C. Y., Y. Y. Chao, M. Y. Yang, S. C. Cho, C. Huei Kao, 2009. Na^+ but not Cl^- or osmotic stress is involved in NaCl-induced expression of glutathione reductase in roots of rice seedlings. Journal of Plant Physiology, 166; 1598–1606.
Ibrahim, E.A., 2016. Seed priming to alleviate salinity stress in germinating seeds. Journal of Plant Physiology, 192; 38–46.
Kaya, C., M. Ashraf, O. Sonmez, S. Aydenir, T.A.L. Una, M.A. Cullu, 2009. The influence of arbuscular mycorrhizal colonization on key growth parameters and fruit yield of pepper plants grown at high salinity. Scient Horticul., 121; 1–6.
Khajeh-Hosseini, M., A.A. Powell, I.J. Bingham, 2003. The interaction between salinity stress and seed vigour during germination of soybean seeds. Seed Science and Technology, 31; 715-725.
Leland, E.F., M.G. Catherine, V.M. Eugene, M.L. Scott, 1994. Time of salt stress affects growth and yield components of irrigated wheat. Agronomy Journal, 86; 100–107.
Levitt, J., 1980. Responses of plants to environmental stresses. Water, radiation, salt, and other stresses. Academic Press, NewYork.
Li, R., F. Shi, K. Fukuda, Y. Yang, 2010. Effects of salt and alkali stresses on germination, growth, photosynthesis and ion accumulation in alfalfa (Medicago sativa L.). Soil Science & Plant Nutrition, 56; 725-733.
Maas, E.V., G.J. Hoffman, 1977. Crop salt tolerance-current assessment. J. Irrig. Drain. Div., 103; 115-134.
Maathuis, F.J.M., A. Amtmann, 1999. K^+ nutrition and Na^+ toxicity: The basis of cellular K^+/Na^+ ratios. Annals of Botany, 84; 123-133.
Mhadhbi, H., V. Fotopoulos, P.V. Mylona, M. Jebara, M.E. Aouani, A.N. Polidoros, 2013. Alternative oxidase 1 (Aox1) gene expression in roots of Medicago truncatula is a genotype-specific component of salt stress tolerance. Journal of Plant Physiology, 170; 111-114.
Munns, R., 2002. Comparative physiology of salt and water stress. Plant Cell Environ., 25; 239-250.
Munns, R., M. Tester, 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59; 651–681.
Neres, M.A., D.D. Castagnara, E.E. Mesquita, M.A. Zambom, L.C.D. Souza, P.S.R.D. Oliveira, C.C. Jobim, 2010. Production of alfalfa hay under different drying methods. Revista Brasileira de Zootecnia, 39; 1676-1683.
Parida, A.K., A.B. Das, 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety, 60; 324–349.
Poustini, K., A. Siosemardeh, M, Ranjbar, 2007. Proline Accumulation as a response to Salt stress in 30 wheat (Triticum aestivum L.) cultivars differing in salt tolerance.' Genetic Resources and Crop Evolution., 54; 925-934.
Roy, S.J., S.J. Negrao, S.M. Tester, 2014. Salt resistant crop plants. Current Opinion in Biotechnology, 26; 115–124.
Sairam, R.K, P.S. Deshmukh, D.S. Shukla, 1997. Tolerance to drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. J. Agron. Crop Sci., 178; 171-177.

Sarruge, J.R.S, H.P. Haag, 1974. An´alises qu´ımicas em plantas, USP-ESALQ, Piracicaba, 56p.

Shabala, S., L. Shabala, E. Van Volkenburgh, 2003. Effect of calcium on root development and root ion fluxes in salinized barley seedlings. Functional Plant Biology, 30; 507–514.

Shannon, M.C, C.M. Grieve, 1999. Tolerance of vegetable crops to salinity. Sci. Hortic., 78; 5–38.

Smart, R.E., G.E. Bingham, 1974. Rapid estimates of relative water concentration. Plant physiology, 53; 258–260.

Szabados, L., A. Savouré, 2010. Proline: a multifunctional amino acid. Trends in plant science, 15; 89-97.

Tavakkoli, E., P. Rengasamy, G.K. McDonald, 2010. High concentrations of Na^+ and Cl^-ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. Journal of Experimental Botany, 61; 4449-4459.

Testerink, C., 2014. Root morphology changes upon salt stress. Plant Physiology Preview, 30; 1-39.

Wang, M, Q. Zheng, Q. Shen, S. Guo, 2013. The Critical Role of Potassium in Plant Stress Response. Int. J. Mol. Sci., 14; 7370-7390.

Wang, W.B., Y.H. Kim, H.S. Lee, K.Y. Kim, X.P. Deng, S.S. Kwak, 2009. Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. Plant physiology and Biochemistry, 47; 570–577.

Zheng, Y., A. Jia, T. Ning, J. Xu, Z. Li, G. Jian, 2008. Potassium nitrate application alleviates sodium chloride stress in winter wheat cultivars differing in salt tolerance. J. Plant Physiol., 165; 1455–1465.