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Impact of salinity and calcium stress on some properties of Isabgol (*Plantago ovata)*

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Abstract

To investigate the influence of salinity and calcium (Ca) on some properties of *Plantago ovata*, a pot factorial experiment based on randomized complete block design with three replicates was conducted in Jiroft, south of Kerman Province, Iran. Three salinity levels of 0, 100 and 200 mM NaCl and Ca levels of 0, 5 and 10 mM Calcium nitrate were applied as the first and second factors, respectively. Results showed that salt levels significantly affected plant height, dry weight, number of spikes per plant, spike length, number of seeds per spike, mucilage and 1000 seed weights. Maximum values for the measured variables were obtained in control and the minimum values in 100 mmol sodium chloride. The effect of Ca on these traits was significant as well; it diminished the adverse effects of salinity. In addition, the interactive effects of salt and Ca on all the above traits were remarkable except for mucilage and spike length on which no significant effect was observed.

Keywords: Calcium nitrate; Jiroft; Mucilage; Sodium chloride; Stress

1. Introduction

Humans have long used herbal medicinal products for healing pains, but plants are able to produce secondary metabolites as a mechanism to cope with a variety of environmental phenomena for their protection and conservation. Thus, when exposed to different ecological conditions, a plant alters its secondary metabolites both qualitatively and quantitatively for adaptation. Therefore, looking at the bases for chemical syntheses in nature and plant species derivation approaches, expansion and diversity in different natural conditions, it is perceptible that production of effective substances in plants is subjected to stresses, environmental conditions and the species adaptability. The effect of environmental factors on plant and its metabolites can be gradual or immediate; living and non-living factors (water, soil and weather) undoubtedly cause immediate changes in plants chemical substances so as to convert into genetic and transferable traits in the long run. Consequently, proper use of environmental conditions is conducive for an increase in the quantity and quality of secondary metabolites and economic efficiency of medicinal herbs in field conditions (OmidBaigi, 2009).

Plantago ovata is a medicinal herb cultivated worldwide. It is an annual grass belonging to the *Plantago* genus and Plantaginaceae Family and a precious plant with a high rate of mucilage from seeds. What makes it a special choice for cultivation as a medicinal herb is its high compatibility with environmental factors. Crop selection in Iran is characterized by specific climatic conditions, water deficiency. Soil susceptibility to erosion and degradation is highly important, especially in arid and semi-arid regions. (OmidBaigi, 2009).

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About 50 percent of Iranian farmlands are exposed to salinity and water deficiency also aggravates this condition. In most parts of the world (Iran), saline soils and irrigation waters are common factors limiting plants growth. From the total farmlands under cultivation throughout the world, 23% are saline (0.34 billion hectares) and 37% sodic (0.54 billion hectares) (Tanji, 1990). This issue is particularly intensified in arid and semi-arid areas where saline waters are used for irrigation (Rangel, 1992).

Identification of salt-tolerant crop cultivars s and their yield after cultivation with saline waters is crucial. During salinity stress, in addition to reduction in water absorption, accumulation of some ions at high concentration levels in plant tissues probably leads to toxicity or ionic imbalance. Due to the dominant presence and abundance of Na^+ and Cl^- in salty waters and soils, there is a reduction in nutrient uptake by plants. As a result, the ratio of sodium ions concentration to calcium and potassium increases. Simple compound alcohol sugars are produced as osmotic regulators to resist salinity stress. The physiological action of these sugars inhibits the binding between adjacent membranes via conserving lipids for cell membrane fluidity during the stress period. One way to understand plant resistance against salinity stress is to examine alterations in its protein content. Salinity stress causes disruptions in plants nutrient balance, changes protein quality and ultimately slows down vegetative growth in plants (Marschner, 1995). Salt poisoning occurs as a result of osmotic stress, nutrients shortage stress and destruction of cell membrane or changes in enzyme activity and metabolism. Salt accumulation indirectly destabilizes soil nutrients, stimulates microorganisms and degrades soil physical structure. Naturally, all these factors influence plant growth and the extent to which a plant is subjected depends on its susceptibility and tolerance. In all, interactions among a variety of factors linked to plant, soil, water and environment determine a plant's response to salinity stress (Chabra *et al*., 1999). Under such stress, plants are exposed to two specific circumstances:

A. It is essential that plants absorbs salts such as sodium chloride (NaCl) to regulate osmosis in cells vacuoles and simultaneously keep NaCl in the cytoplasm (which is the pathway for the movement of salt to vacuoles) at a low concentration.

B. The uptake of excess NaCl as a result of transpiration must be efficiently impeded in order to prevent its damaging accumulation in plant cells, tissues and organs. Also, salt uptake in leaves must be controlled; any salt present in the water permeates into leaves through the xylem and can return to the roots via the phloem, but the latter is not very efficient (Glenn *et al.*, 1997).

Root cell membranes have low permeability to sodium, yet the same cells can absorb potassium moving against the concentration gradient. The necessity of calcium for keeping cell membranes in health has also been proven (Francois et al., 2001). The most frequent activity of calcium is related to its ability in coordinate binding which creates a stable but reversible binding between molecules. Such actions are most often performed in plant cell walls and plasma membrane (Marschner, 1995).

Salinity affects Ca^{+2} uptake and transfer; therefore, plants display symptoms of Ca deficiency, especially in sensitive genotypes (Francois et al., 2001). Since Na^+ in plasma membrane is replaced by Ca^{2+}, a high ratio of Na^+:Ca^{2+} binding to the apoplast outside the plasma membrane may exert an influence on membrane functions (Schroeder and Thuleau, 1991).

Adding Ca^{2+} to the plant's environment decreases the extent of salt damage by a reduction in Na^+ ions toxicity, more efficiently than neutralizing osmotic effects associated with salinity stress. With the addition of calcium, the disruptions to cellular calcium homeostasis caused by Na^+ become ineffective. Furthermore, adding calcium by making changes in the uptake rate to the benefit of K^+ alters the K^+: Na^+ ratio; cellular membranes coordination and enhancement by Ca^{2+} decreases root cells leakage and a combination of all these factors increase K^+: Na^+ ratio (Cramer *et al*., 1994).

Some researchers have examined the impact of different salinity levels on Isabgol germination (Haghighi and Milani, 2009; Hosseini and Moghadam, 2006; Dadkhah and Kafi, 2012). In one study, effects of various levels of calcium carbonate on two cultivars of sorghum were investigated under saline irrigation in the field and in a greenhouse. The findings showed that with increased salinity rates, there was a decrease in *Plantago psyllium* traits (bush height, leaf area, leaf dry weight, stem, root and biomass, ratio of aerial organs, weight to root and ratio of leaf weight to stem). In contrast, with the elevation of

calcium carbonate rates at any salinity level, there was a dramatic increase in the above traits (Yarnia, 2006). *Plantago ovata* morphological properties were also studied at different salinity rates. The result showed that with increased salinity, there was a decline in the parameters associated with the plant's morphological growth (Safarnejad *et al*., 2007).

Thus far, no report has been published on the effect of calcium and salt on *P. ovata* in its vegetative period as studies have been more focused on effects on germination. Thus, the main objective of this research is to examine the impacts of NaCl and calcium and their interactive effects on the yield and yield components of *P. ovata.*

2. Material and methods

2.1. Time, location and climatic conditions of the experiment

The experiment was performed at the Agricultural Research Field of the Islamic Azad University in Jiroft, South of Kerman Province, Iran in 2012. The region has a longitude, latitude and altitude of 57° 32′E, 28° 32′N and 675 m above sea level, respectively. The climate is arid and semi-arid with an average annual rainfall of 175 mm and relative humidity of 50-60%. The usual maximum and minimum temperatures are 49 and 1 °C, which rarely dips by -2 °C.

2.2. Characteristics of the experimental design

A sample was taken to obtain and measure soil characteristics and some physical and chemical properties. (Table 1). This experiment was conducted in a factorial form based on randomized complete block designs with 3 three replicates. Three salinity levels (0, 100 and 200 mM sodium chloride) and calcium nitrate levels (0, 5, 10 and 15 mM) were taken into account as the first and second factors. Treatments were applied after the 2-leaf stage, after which the plants were irrigated with calcium nitrate-containing saline water. 3 kg of the soil sample was poured into each pot and Isabgol seeds were planted inside; each pot contained 10 seeds placed at the 1cm depth. For measuring the properties at the end of the growth season, 5 bushes were selected from each pot and then bush length, bush dry and wet weights, 1000 seed weight, spike length, number of spike per plant and number of grains per spike were measured.

This research applied the Kalian Son Drum method to determine the rate of mucilage in seeds (Ebrahimzadeh *et al*., 1997). Data were collected and then analyzed using SAS—the statistical analysis software system. Means were compared with the Duncan's multiple range test at 5% significance level.

Table 1. Some chemical and physical properties of the studied soil

Available K (ppm)	Available P (ppm)	O.C (%)	Total N (%)	pH	EC (dS/m)	Soil Texture
233.4	5.6	0.156	0.026	7.5	1.21	Sandy Loam

3. Results and discussion

3.1. Plant Height

According to variance analysis, calcium had a significant effect on bush height at 1% level (Table 2). A maximum height of 17.46 cm was gained after application of 15 mM calcium and a minimum of17.17 cm when calcium was not applied (Table 3). As the study indicates, Ca application plays a pivotal role in increasing plant height. Most Iranian soils contain high calcium contents. However, since Ca mobility and transport is low in plants, they show a positive response to increased amounts. Increase in bush height can also result from improved cellular metabolism and increased cells growth in the presence of calcium ions. Yarnia (2006) reported an increase in sorghum height due to increased application of calcium carbonate. Results from variance analysis showed that the effect of salinity stress on bush height was significant at 1% level (Table 2). Maximum and minimum heights of 17.78 and 16.87 cm were achieved by no-salinity stress and 200 mM salinity, respectively. With increased salinity, there was a remarkable decrease in bush height (Table 4). Salinity causes deficiency in plant water by raising soil solution osmotic stress. As water is a major factor in plant growth, its shortage reduces plant height. The interactive effect of Ca and salt stress on plant height was significant at 1% (Table 2): plant height reached a peak of 18.19 cm in the absence of salinity stress and simultaneous application of 15 mM calcium; the lowest height was 16.80 cm with the application of 5 mM Ca under 200 mM

salinity stress (Table 5). Based on our findings, calcium reduces the adverse effects of salinity, induces an increase in potassium uptake and prevents the intake of sodium. Its specific role in maintaining cell membrane coordination reduces the negative effect of salts on plant growth. In a study by Panah and Zadeh (2005), the addition of sodium chloride reduced potassium intake in rootlet and plumule and in contrast, increased sodium within. However, calcium nitrate application caused a decline in the adverse effects of salinity.

Table 2. Results for Data Variance Analysis of Isabgol Properties

s.o.v	df	Spike Length	1000 Seed Weight	Grain number per Spike	Spike Number per Plant	Dry Matter	Height	Mucilage
Calcium (a)	3	0.001^{**}	0.001^{**}	3.77^{**}	0.25^{**}	0.09^{**}	0.13^{**}	0.02^{**}
Salinity (b)	2	0.06^{**}	1.01^{**}	224.55^{**}	75.03^{**}	11.33^{**}	2.52^{**}	0.91^{**}
a.b	6	0.00002^{ns}	0.0004^{**}	2.54^{**}	0.03^{*8}	0.004^{**}	0.45^{**}	0.003^{ns}
Error	24	0.00001	0.00004	0.00078	0.001	0.0008	0.001	0.0002
CV(%)		1	1.44	2.5	3.2	4.2	5.3	5.2

(ns= no significance; ** Significance at 1% probability level)

The findings of this research are in agreement with Yarnia's (2005) study which showed that plant growth and height declined with increased salinity and calcium ions play a vital role in minimizing salinity effects.

Table 3. Mean comparison of calcium treatments for Isabgol properties

Calcium	Spike length (cm)	1000 Seed weight (g)	Grain number per Spike	Spike number per plant	Plant dry matter (g)	Height (cm)	Mucilage (%)
0	1.83^{d}	1.53^{d}	32.16^{d}	13.1^{d}	2.37^{d}	17.17^{b}	44.88^{d}
5 Mm	1.84^{c}	1.55^{c}	32.28^{c}	13.18^{c}	2.41^{c}	17.25^{c}	40.92^{c}
10 Mm	1.85^{b}	1.56^{b}	32.39^{b}	13.31^{b}	2.5^{b}	17.34^{b}	40.97^{b}
15 Mm	1.86^{a}	1.58^{a}	32.61^{a}	13.49^{a}	2.6^{a}	17.46^{a}	41^{a}

Means with thesame letters in each column are not significant at 5%.

Table 4. Mean comparison of salinity treatments for different Isabgol properties

Salinity	Spike length (cm)	1000 Seed weight (g)	Grain number per Spike	Spike number per plant	Plant dry matter (g)	Height (cm)	Mucilage (%)
0	1.91^{a}	1.75^{a}	36.25^{a}	15.67^{a}	2.79^{a}	17.78^{a}	41.26^{a}
100 Mm	1.86^{b}	1.7^{b}	32.46^{b}	13.46^{b}	2.45^{b}	17.27^{b}	40.81^{b}
200 Mm	1.77^{c}	1.22^{c}	27.62^{c}	10.68^{c}	2.17^{c}	16.87^{c}	40.76^{c}

Means with thesame letters in each column are not significant at 5%

Table 5. Mean comparison of interactive effect of Ca and salt on different Isabgol properties

Treatment (Calcium, Salinity)	Height (cm)	Dry matter (g)	1,000 seed weight (g)	Spike number Per plant	Grain number per Spike
(0, 0)	17.58d	2.69d	1.72cd	15.41d	36.09d
(0, 100)	17h	2.32g	1.68e	13.34g	29.92h
(0, 200)	16.95h	2.1j	1.21g	10.56j	27.46l
(5, 0)	17.88c	2.76c	1.73c	15.5c	36.19c
(5, 100)	17.06g	2.35f	1.69f	13.45f	33.1g
(5, 200)	16.88j	2.12j	1.22fg	10.6j	27.55k
(10, 0)	18.07b	2.82b	1.76b	15.72b	36.26b
(10, 100)	17.12f	2.49f	1.71d	13.49f	33.35f
(10, 200)	16.83ij	2.18i	1.22fg	10.71i	27.65j
(15, 0)	18.19a	2.88a	1.79a	16.04a	36.44a
(15, 100)	17.34e	2.63e	1.71d	13.58e	33.58e
(15, 200)	16.89i	2.29h	1.23f	10.85h	27.8i

Means with thesame letters in each column are not significant at 5%

3.2. Dry Weight

Calcium showed a significant effect on plant dry weight at 1% level (Table 2). The highest and lowest dry weight values (2.6 and 2.37 g) were obtained from the application of 15 mM calcium and non-use of calcium, respectively (Table 3). With increased calcium application, plant dry weight increased up to a significant point. Calcium is an essential nutrient for plants. As the

soil under study is light, it is possibly exposed to calcium deficiency which eventually affects Isabgol planttherefore the addition of calcium to the soil increases plant dry weight. Even if there is a high level of calcium in the soil, its mobility in the plant is very limited because it occurs mainly in the xylem and its transport via the phloem is almost passive. The results revealed that the plant showed a positive response to a constant supply of calcium. As well as examining the effects of Ca levels on such Isabgol properties as dry weight, anions associated with calcium must be considered. In this research, calcium was sourced from calcium nitrate from which nitrate ions cause a higher calcium uptake.

Salinity stress affected dry weight considerably at 1% (Table 2). Normal conditions (no salinity stress) led to the highest dry weight of 2.79 g and 200 mM salinity stress resulted in the lowest value of 2.17g (Table 4).

As Table 5 shows, a reduction in dry weight occurs due to an increase in salinity, which influences plant growth and yield by exerting osmotic pressures and ionic effects. Salinity restricts water uptake and may cause nutrient imbalances and toxicities in plants as well. All of these factors can minimize plant growth and yield.

The interactive effect of calcium and salt stress on dry weight was significant at 1% (Table 2). Maximum dry weight of 2.88 g was obtained from 15 mM calcium under normal conditions (absence of salinity) and a minimum of 2.10 g from 200 mM salt stress (Table 5). The findings of this study indicates that calcium could prevent salt adverse effects and boost plant vegetative growth and yield. Here, salinity was imposed on the plant through NaCl and calcium nitrate; salinity induces nutrient imbalance and toxicity in plants. Saline soils usually contain high concentrations of chloride ions and because chloride is an anion and prevents the uptake of other anions like nitrate, it causes an imbalance in nitrate to chloride ratio in plants. Moreover, chloride ions are present in soil solution and most of it are taken up by plants resulting in chloride toxicity. This study applied calcium with nitrate; the latter inhibits adverse effects of chloride and salinity and elevates plant growth and yield parameters. Yarnia (2005) reported that salinity caused a decrease in sorghum dry weight due to increased calcium carbonate levels.

The findings of the present study are in agreement with those of Safar et al. (2007) and Mozafari and Monchari (2005) who reached the conclusion that salinity restricts production of dry matter in plants and increase in calcium diminishes the adverse effects of salinity..

3.3. Number of spike per plant

According to variance analysis, calcium application significantly affected number of spike per plant at 1% level (Table 2). An application of 15 mM calcium resulted in the highest value of 13.49 in the number of spike; without calcium, the lowest number of spike was 13.10 (Table 3). The number of spike increased significantly with increased calcium.

At different salinity levels, there was a considerable difference between the numbers of spike per plant at 1% level (Table 2). Under normal conditions (without salinity), maximum number of spike was 15.67, whereas extreme salinity (200 Mm) resulted in a minimum of 10.68 (Table 4).

The interactive effect of salinity and calcium on number of spike was also significant (Table 2). At 15 mM calcium application under normal conditions (no salinity), the utmost number of spike per plant was 16.04. However, no calcium application and application at 5 mM under increased salinity stress resulted in the lowest numbers of spike of 10.6 and 10.56, respectively (Table 5).

3.4. Number of seeds per spike

The effect of calcium on number of grain per spike was significant at 1% level (Table 2). The highest number of grain per spike (32.61) resulted from 15 mM calcium application and the lowest (32.16) from no-use of calcium (Table 3). At 1% level of significance, 1 a significant increase in the number of grain per spike occured with increased calcium application. Likewise, salinity stress had a great bearing on this trait at 1% level (Table 2). Optimum number of grain per spike (36.25) were observed in normal conditions (no salinity) and minimum (27.62) from extreme salinity levels (200 mM) (Table 4). Increased salinity stress resulted in decreased number of grain per spike.

Interactive effects of salt stress and calcium on number of grains per spike were so significant that the greatest number of grain per spike (36.44) resulted during no salinity and 15 mM calcium application while the lowest values (27.46)

resulted from extreme salinity (200 Mm) with no calcium application (Table 5).

3.5. The Thousand seed weight

According to Table 2, 1000 seed weight was strongly affected by calcium at the 1% level of significance. Maximum and minimum weights of 1000 seeds (1.58 and 1.53 g) resulted from 15 mM calcium application and no use of calcium, respectively. This is indicates that increased 1000 seed weight increases with increased calcium (Table 3). 1000 seed weight was also subjected to salinity stress at 1% level (Table 2); the greatest and smallest amounts of 1000 seed weight (1.75 and 1.22 g) were observed with no salinity stress (normal conditions) and intense salinity stress (200 mM), respectively. Consequently, there is a marked reduction in 1000 seed weight under salt stress (Table 4).

Interactive effects of salinity and calcium on 1000 seed weight were significant at 1% (Table 2). Optimum weight of 1000 seeds (1.79 g) was obtained from of the application of 15 mM calcium in the absence of salinity while the minimum amount (1.21 g) was observed with non-use of calcium under excessive salt stress (200 mM). As such, it is shown that the plant copes better with salinity in the presence of elevated calcium amounts (Table 5).

Similar to the present work, a number of researches (Mozafari and Kalantari, 2005; Yarnia, 2005) stated that calcium can minimize negative effects of sodium on plant leaves such as reduction in leaf area and leaf drop. Therefore in a saline environment, the adverse effects of increased salinity such as decrease leaf area, leaf weight and grain weight will be countered if the plant is provided with a sufficient amount of calcium.

3.6. Spike length

The effect of calcium on spike length was significant. With application at 15 mM, the highest spike length of 1.87 cm was recorded while with no calcium application, the lowest spike length of 1.83 cm was observed (Table 3). With increased calcium application, spike length showed a significant increase (Table 3).

Under varying salinity stress levels, spike lengths were significant at 1%; the highest spike length (1.91 cm) and the lowest (1.77 cm) resulted from normal conditions (no salinity) and extreme salinity levels (200 mM), respectively. Increased salinity levels results in significantly decreased spike length (Table 4).

No significance was observed regarding the interactive effects of salt stress and calcium on spike length (Table 2).

3.7. Mucilage

Like other traits, mucilage was subjected to calcium at 1% probability level (Table 2). Optimum and minimum mucilage percentages (41 and 40.88%) were achieved with 15 mM calcium application and ano-calcium application, respectively (Table 3). There was a substantial increase in mucilage percentage with increased calcium application (Table 3).

Salinity stress had a significant effect on mucilage percentage at 1% (Table 2). The highest and lowest mucilage percentages (41.26 and 40.76 %) were obtained under normal conditions of no-salinity and severe salt stress (200 mM), respectively (Table 4). Salt stress therefore, caused mucilage to decline significantly.

No significance was observed with the interactive effects of calcium and salinity stress on mucilage, showing.

4. Conclusion

The results of the present study indicate that maximum yield, yield components and mucilage increased under normal conditions without salinity and 15 mM calcium application and are at a minimum from severe salt stress under absence of calcium, indicating the pivotal role of Ca in improving plant tolerance to salinity stress. In summary, vegetative traits and yield components of *Plantago ovata* increased with the application of calcium at different rates under no-saline conditions. At low salinity levels, all rates of calcium application increased vegetative growth and yield of the plant. However, at higher levels, there was a need for higher calcium application rates to minimize the adverse salinity stress.

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