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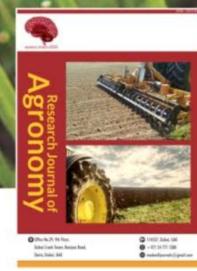

Study of Salinity Stress and Calcium Interaction on Morphological and Physiological Traits of Vicia Villosa under Hydroponic Condition

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Key words: Vicia villossa, salinity stress, calcium, Hydroponic

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Abstract: For the study of salinity stress on Vicia villosa and calcium effect for modulation of that, it was conducted an experiment under hydroponic condition and valuated some important morphological and physiological characteristics. This experiment was conducted as factorial based on randomized complete design with three replications. The treatments include salinity stress in 3 levels (0, 50 and 100 Mm NaCl) and calcium in 2 levels (content in Hoagland solution and double content). The results showed that all of morphological and physiological traits include root and shoot length, root and shoot wet and dry weight, leaf area, leaf chlorophyll content, RWC, CMS and biological yield had significant difference with control and affected by salinity stress severity. But, calcium effect on them was not significant, despite of decreasing salinity effect.

INTRODUCTION

The salinity is the excessive concentration of mineral elements in the water or soil solution which causes the accumulation of salt in the root zone and the plant is struggling to absorb enough water from the soil solution. The salinity is included abiotic environmental stress that it can affect plant growth and is the most important factor limiting agricultural production in arid and semi-arid regions[1, 2]. It is estimated that >300 million hectares of cultivated lands and close to half irrigated lands have been affected by salinity[3]. The severe salinity stress drastically reduces plant growth which is due to osmotic stress and mineral absorption imbalance and toxicity of the special ionic imbalance[4]. The salinity stress by reducing the water potential of roots cause to decrease the ability of the water uptake by plant roots, also the increase of salinity in the root increase absorption and transport of toxic ions in plant tissues which reduces the absorption of essential elements and disturbe the ion balance and cause toxicity from accumulations of sodium and chlorate ions[5].

Calcium in processes of the maintaining of membrane structure, ion exchange regulation and controlling of ions interchanges plays an important role[6]. It has been reported that salinity reduced the dry weight of rice, while adding calcium cause to increase the shoot dry weight[7]. The most important strategies of plants for resistance against the stress is the accumulation of compatible solutions, such as can be noted soluble sugars[8]. Soluble sugars are a group of compatible osmolytes that accumulate under salt stress and act as osmotic protector. Osmotic regulators, such as glucose and fructose and sucrose and polyoses are as protecting factors of plants against abiotic stress[5]. Calcium form intercellular connections that preserve structure and integrity of cell membranes and cell walls. Calcium operates as a second messenger in signal transmission path in the cell. One of the solutions to avoid salinity effects is the using of calcium[9]. Calcium is absorbed from root tip as the non-active transfer and through symplastic and apoplastic pathways imports into the roots and transfers to aerial parts of the plant via the xylem[10]. Calcium regulates the absorption of nutrients across the plasma membrane and has roles for the plant cell division and elongation and in the structure and permeability of cell membranes and in nitrogen metabolism and carbohydrates displacement[11].

Photosynthesis is one of the important physiological processes that are influenced by genetic and environmental factors. Due to salinity, the chlorophyll content decreases which this can be resulted by the decrease of chlorophyll synthesis and its destruction. The destruction of the chlorophyll molecule is as a result of the phytol chain separation from porphyrin ring by oxygen free radicals or chlorophyllase enzyme. Due to salinity, oxygen free radicals increase in chloroplasts and cause to increase the destroying chlorophyll molecules and chloroplast membrane which leads to reduce photosynthesis and growth. Today, due to the indiscriminate use of natural resources and the use of inappropriate technologies in crop production, particularly in relation to irrigation, a significant portion of agricultural lands in arid areas were confronted with salinity phenomena[12].

Hairy vetch is a valuable legume at cold regions with temperate summers. In relation to cover crop and particularly forage used domesticated animals as palatable species in arid and semi-arid regions is very important. Hairy vetch belongs to Papilionaceae family and from the past the different types of its species is cultivated. This plant is as bush with compound leaves. Hairy vetch has a different use cases such as green fodder, hay, pasture, soil improvement or green manure, preventing the erosion especially on wet sandy soils or intercropping with crops of barley especially.

In this study, the effect of different levels of salinity and calcium on the growth and morphological and some physiological characters of hairy vetch in a hydroponic system are studied.

MATERIALS AND METHODS

Plant material: In this study, a genotype of hairy vetch was used.

Hydroponic culture: In this study, a static culture system ventilated was used. The experiment was conducted in a greenhouse equipped with heating and lighting systems. Greenhouse settings included 40% relative humidity; temperature at light period 3±20°C; temperature at dark period 3±16°C and photo period was 16 hrs of light and 8 h of darkness. The seeds are disinfected with sodium hypochlorite 1% for 15 min and then to germinate uniformly, seeds were planted in wet filter paper in Petri dishes and were maintained at the growth chamber at 25°C and 60% humidity. After germination and rootlet production, seedlings were transferred into holes within 5×5 cm from each other on a floating polystyrene plastic containers measuring 18×27×37 cm when were inside the tubes with net at the their bottoms. Hoagland nutrient solution was used for the nutrition of seedlings and after a week, salinity and calcium treatment were applied. Each pot contains 10 L of nutrient solution and nutrient solution was replaced in suitable time interval. During the test, the containers of seedlings in good conditions for optimal ventilation were permanently aerated.

Treatments in hydroponic system: A factorial experiment in the completely randomized design with three levels of salinity (control, 50 and 100 mM) and two levels of calcium (the amount in Hoagland solution and two times of it) were performed in three replications. The salinity stress was applied after ten days. To prevent osmotic shock, NaCl was added to dishes containing nutrient solution every three days, until finally the desired concentrations in the culture medium were achieved[13, 14]. Three weeks after salinity stress, sampling of seedlings of hairy vetch was conducted to investigate the effects of stress on genotypes and the following characters were measured.

Studied characters

Root and shoot length: After removing the roots from the seedling, root and shoot length were measured using a ruler.

Root and shoot fresh weight: The fresh weight of root and shoot were achieved using a digital scale with accurate 0.0001.

Root and shoot dry weight: First, samples were put in an oven with temperature 72°C for 48 h and then were measured with a digital scale with accurate 0.0001.

The biological yield: The biological yield was calculated from the sum of root and shoot dry weight.

Chlorophyll content: Chlorophyll content in seedling stage and three weeks after the stress using chlorophyll meter (model SPAD-502) for the young and developing leaves and mean values (4 data) were recorded. The device automatically by measuring the absorption spectrum in the range of blue light (400-500 nm) and the red light range (500-600 nm) measure the amount of chlorophyll as non-destructive[15].

Cell Membrane Stability (CMS): The leaf samples were randomly taken from each treatment and were immediately washed with distilled water. The leaves were divided into two equal parts and one part was placed in a solution without the PEG and another in a solution with 40% PEG solution for 24 h at 25°C. After this period, the first EC reading was conducted. After EC measurement, all leaves at a temperature of 120°C and the pressure of 1 atmosphere were autoclaved for 20 min and after the cooling of solutions and get to 25°C, the second readings were recorded. The membrane stability was calculated using the following Eqution:

$$CMS = \frac{1-\frac{T_1}{T_2}}{1-\frac{C_1}{C_2}}$$

where, T_1 and T_2 are the first readings of EC for treatment under stress with PEG and control and C_1 and C_2 are the second readings for treatment under stress with PEG and control, respectively.

Leaf Relative Water Content (RWC): To measure this character, leaf samples were randomly taken and immediately their Fresh Weights (FW) were measured by the digital scale. The samples were placed in an oven at 70°C for 72 h and were weighed again (DW). Finally, using the following formula, RWC was calculated by percentage:

$$RWC = \left\{\frac{FW\text{-}DW}{FW}\right\} \times 100$$

RESULTS AND DISCUSSION

The results of variance analysis showed that were significant differences between different levels of salinity in terms of membrane stability, chlorophyll content, leaf area, stem length, root dry weight, shoot dry weight and biological yield (p = 0.01) and the relative water content of leaf and root length (p = 0.05). Two levels of calcium did not statistically show any significant difference for all indices measured. Calcium partly reduces the negative effects of stress but its effects were not significant statistically. Also, the interaction of the calcium and salinity was not significant for any of the measured parameters (Table 1).

Mean comparison of the different levels of salinity showed that increasing the severe of salinity stress reduced the stem length, root length, shoot dry weight, root dry weight, leaf area and biomass. Most amounts of these characters belong to the control treatment and these characters decreased with increasing of salinity. So that, the least of these characters was observed in NaCl 100 mM. The most common adverse effect of salinity on most plants is the reducing of plant height and the quality of its products. Therefore, when plant shoot growth has the severe decline under salinity stress it damages to the final performance of plant irreparable[16]. Disorder at plant growth and its loss under salinity stress is due to the decreasing of photosynthetic level[17]. Previous studies have shown that salinity stress in wheat and barley cause to decrease stems length and plant height[18].

Increasing salinity has also made to reduce root length, so that the maximum root length with an average of 37.81 mm and the minimum its length with an average of 28.94 mm were observed in the control and 100 mM salinity treatments, respectively (Fig. 1). Salt concentration directly affects the growth stages of plants. The lack of ionic and osmotic balance is considered among the destructive effects of salinity and root is the first limb that due to the absorption of elements is directly confronted with stress[18]. Rooting is among parameters that are severely affected by salinity, as studies have shown, salinity has made to decrease root length in tomatoes[19] and potatoes[20]. Other effect of salinity stress has been a decrease in stem length. The highest stem length with an average of 41.5 mm and the lowest its length an average of 35.1 mm were observed in the control and 100 mM salinity treatments, respectively (Fig. 1). Shoot dry weight dramatically decreases with increasing salt concentration which similar results in Qolam etc, studies have also been obtained. Ion toxicity resulting from an increase in salinity is caused to impair all over the biological and metabolical activities of plants, leading to an intense reduction or loss of shoot[21].

Table 1: Analysis of measured characters

S.O.V	df	RWC	CMS	chlorophyll	Leaf area	Root length	Shoot length	Root dry weight	Shoot dry length	Biological yield
Salinity	2	*356.91	**1236.44	**115.25	**31.32	*118.06	**61.46	**216.50	**822.06	**1866.72
Calcium	1	0.12 ns	67.84 ns	**165.16	0.003 ns	28.25 ns	5.14 ns	28.75 ns	43.56 ns	1.53 ns
calcium×salinity	2	1.73 ns	18.86 ns	4.00 ns	0.64 ns	3.59 ns	1.19 ns	19.68 ns	45.01 ns	45.12 ns
Error	12	72.718	49.77	10.01	2.16	31.39	2.96	10.62	96.36	115.32
CV%	-	10.28	9.31	15.18	22.63	16.80	4.49	10.76	15.15	11.29

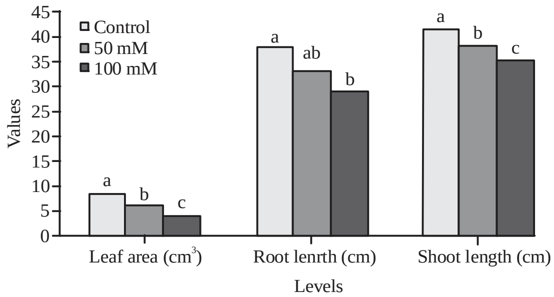

Fig. 1: The effect of different salinity levels on leaf area, root and stem length

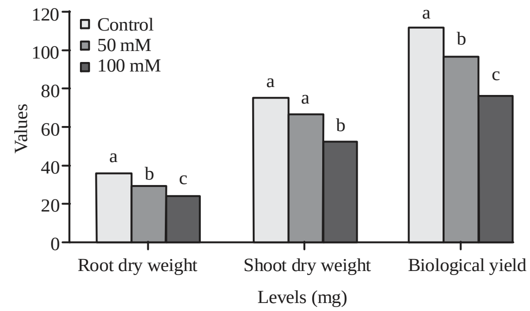

Fig. 2: The effect of different salinity levels on biological yield, shoot and root dried weight

Resulting from the comparison of the means showed that an increase in the intensity of salinity stress also decreased plant leaf area which can be due to reducing the absorption of water by the roots and consequently reducing the amount of water in the aerial parts of plant. As previous characters, the greatest leaf area with an average of 8.84 cm^3 and the least leaf area with an average of 4.28 cm^3 were observed in the control and 100 mM salinity treatments, respectively (Fig. 1).

The results showed that increasing salinity has decreased shoot dry weight, so that the maximum shoot dry weight (36.46 mg) and the lowest of that (24.46 mg) were observed in the control and 100 mM salinity treatments, respectively. Adding calcium to nutrient solution was caused to reduce the negative effects resulting from salt stress but this effect was not statistically significant (Fig. 2). This decline could be due to large amounts of harmful ions such as Na^+ and Cl^- and disorder in the metabolism of other nutrient elements caused by the ions[6].

The different levels of Salinity had a significant effect on root dry weight, so that increasing the intensity of stress decreased root dry weight. The highest root dry weight (36.46 mg) and the lowest of that (24.46 mg) were observed in the zero and 100 mmol salinity levels, respectively. Decreasing root dry weight as other characters results from the negative effects of salinity stress which is caused to reduce the production of roots and stems by embryo. The roots absorb water and minerals and salinity stress arrives to the plants via roots. Therefore, the root is the first limb that is confronted with stress. Plants for making the organic materials such as proline, mannitol, glycine betaine and sorbitol as well as osmotic adjusting to deal with salinity consume much energy. Thereby, the efficiency of roots decreases to provide nutrient elements and water for other plant organs, causing to reduce shoot growth. As a result, salinity stress is caused to decrease organogenesis and dry matter production and the transfer of nutrients from cotyledons to the embryonic axis and finally the root and stem weight.

Biological yield reduced due to increasing the concentration of calcium chloride, so that the maximum amount of that (111.87 mg) at salinity stress level of zero and the lowest of that (76.71 mg) at salinity stress level of 100 mM was observed (Fig. 2). Reducing photosynthetic levels and the excessive consumption of energy to control and reduce the effect of salinity stress and making the ionic and osmotic balance to avoid the toxicity of ions as well as to maintain cell swelling can be of the major factors of decreasing function in many plants. Studies on wheat, barley and lentil showed that increasing salinity stress decreases biological yield[14].

The results showed that increasing the intensity of salinity stress decreases the amount of chlorophyll on leaves. With increasing the intensity of stress to 50 and 100 mM, the amount of chlorophyll on leaves also decreased 22.5 and 33.5%, respectively. The highest amount of chlorophyll on leaves with an average of 26.63 at salinity stress level of zero and the lowest of that with an average of 17.03 at salinity stress level of 100 mM were observed (Fig. 3). The amount of chlorophyll on leaves, as one of the key factors for determining of photosynthesis and dry matter production, is of great importance. Reducing the amount of chlorophyll can be had a great impact on the photosynthetic activity and dry matter production. A significant reduction of chlorophyll in the high concentrations of salinity stress is due to tissue destruction in chloroplasts[22]. Rao and Rao[23] reported that the destruction of chlorophyll in the high concentrations of salinity stress results from increasing chlorophyll enzymes and minerals in chloroplasts which would add ions to the chlorophylls.

In this test with the exercising of salinity stress, relative water content on leaves as other indicators measured had been decreased. The highest relative water content on leaves with an average of 91.3% at salinity stress level of zero and the lowest of that with an average of 76.09% at salinity stress level of 100 mM were observed (Fig. 3).

Table 2: Regression of studied characteristics

Characters	RWC	CMS	Chlorophyll	Leaf area	Root length	Shoot length	Root dry weight	Shoot dry weight	Biological yield
RWC	1								
CMS	**0.666	1							
Chlorophyll	*0.525	*0.560	1						
Leaf area	*0.461	**0.714	*0.527	1					
Root length	0.085ns	0.360ns	0.305ns	0.459ns	1				
Shoot length	*0.554	**0.791	*0.476	**0.784	**0.588	1			
Root dry weight	**0.598	**0.786	*0.535	**0.762	**0.581	**0.725	1		
Shoot dry weight	0.382ns	**0.588	*0.522	**0.768	*0.487	**0.754	**0.606	1	
Biological yield	*0.494	**0.715	*0.577	**0.841	*0.567	**0.817	**0.802	**0.961	1

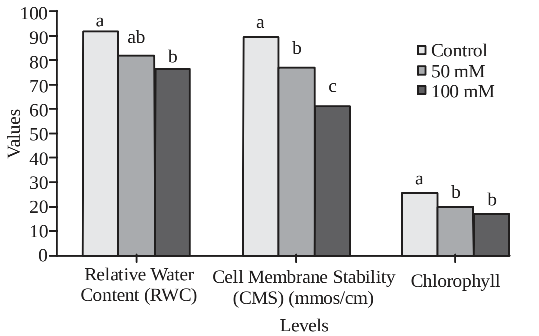

Fig. 3: The effect of different salinity levels on RWC, CMS and chlorophyll

The results showed that increasing the intensity of salinity stress decreased cell membrane stability. The highest cell membrane stability (89.64 mmos cm^{-1}) at salinity stress level of zero and the lowest of that (60.97 mmos L^{-1}) at salinity stress level of 100 mM was observed (Fig. 3).

The study of simple correlation among characters showed that there is a highly significant correlation between the most of them with the exception of root length that had no significant correlation with relative water content on leaves, cell membrane stability, the amount of chlorophyll and leaf area. Furthermore, the correlation between shoot dry weight and relative water content on leaves was not significant (Table 2). Among the characters, plant biological yield had a highly correlation with most characters and the highest amount of that was related to the correlation of this character with the shoot dry weight (r = 0.96) and as mentioned above, root length has a little correlation with other characters and the lowest of that has been associated with the amount of chlorophyll on leaves (r = 0.30).

CONCLUSION

Also, Askari *et al.*[24] in their study reported that chlorophyll and membrane stability index had a positive and significant correlation with the yield. Identifying physiological characters affecting the yield has been mentioned as one of the most important ways of modifying the yield.

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