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

Raheleh Khademian, Roghayeh Aminian

Abstract—For the study of salinity stress on *Vicia villosa* and calcium effect for modulation of that, an experiment was conducted under hydroponic condition, and some important morphological and physiological characteristics were evaluated. This experiment was conducted as a factorial based on randomized complete design with three replications. The treatments include salinity stress in three levels (0, 50, and 100 mM NaCl) and calcium in two levels (content in Hoagland solution and double content). The results showed that all 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 was significantly different from the control and is affected by the salinity stress severely. But, calcium effect on them was not significant despite of decreasing salinity effect.

Keywords—*Vicia villossa*, salinity stress, calcium, hydroponic.

I.Introduction

THE salinity which is the excessive concentration of mineral elements in the water or soil solution causes the accumulation of salt in the root zone, and the plant is struggling to absorb enough water from the soil solution [26]. The salinity included abiotic environmental stress that can affect plant growth and it is the most important factor limiting agricultural production in arid and semi-arid regions [5], [6]. It is estimated that more than 300 million hectares of cultivated lands and close to half irrigated lands have been affected by salinity [21]. The severe salinity stress drastically reduces the plant growth, which is due to osmotic stress, mineral absorption imbalance, and toxicity of the special ionic imbalance [15]. The salinity stress, by reducing the water potential of roots, causes to decrease the ability of the water uptake by plant roots, and also, the increase of salinity in the root increases absorption and transport of toxic ions in plant tissues, which reduces the absorption of essential elements and disturbs the ionic balance and causes toxicity from the accumulations of sodium and chlorate ions [19].

During the processes such as the maintenance of membrane structure, ion exchange regulation, and controlling of ions interchanges, calcium plays an important role [18]. It has been reported that salinity reduced the dry weight of rice, while adding calcium causes to increase the shoot dry weight [3]. The most important strategy of plants for the resistance against the stress is the accumulation of compatible solutions such as soluble sugars [25]. Soluble sugars are a group of compatible osmolytes that accumulate under salt stress and act as an osmotic protector. Osmotic regulators such as glucose, fructose, sucrose, and polyoses are the protecting factors of plants against abiotic stress [19]. Calcium forms intercellular connections that preserve structure and integrity of cell membranes and cell walls. Calcium is operated as a second messenger in signal transmission path of the cell. One of the solutions to avoid salinity effects is the use of calcium [23]. Calcium is absorbed from the root tip through the non-active transfer and through the simplistic and apoplectic pathways. It is imported into the roots and transferred into aerial parts of the plant via the xylem [28]. Calcium regulates the absorption of nutrients across the plasma membrane and plays a role for the plant cell division and elongation in the structure and permeability of cell membranes and for the nitrogen metabolism and carbohydrates displacement [8].

Photosynthesis is one of the important physiological processes that are influenced by genetic and environmental factors. Due to the salinity, the chlorophyll content decreases, and this can result in the decrease of chlorophyll synthesis and its destruction. The destruction of the chlorophyll molecule is a result of the phytol chain separation from porphyrin ring by oxygen free radicals or chlorophylls enzyme. Because of the salinity, oxygen free radicals increase in chloroplasts and cause to increase the destruction of chlorophyll molecules and chloroplast membrane, which leads to reduce the photosynthesis and the plant growth [10]. Today, for the reason of 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 is confronted with the salinity phenomena [12].

Hairy vetch is a valuable legume at cold regions with temperate summers. The use of cover crops and forage such as vetch is one of the main factors in sustainable agriculture, which brings the optimal use of resource, human and animal nutrition and reduces the weed population in addition to the increasing yield. Hairy vetch belongs to Papilionaceae family, *Vicia* genus, and almost 150 species of the plants have been identified. Hairy vetch has different functions such as green fodder, hay, pasture, soil improvement or green manure,

Raheleh Khademian, Assistant Professor, is with the Department of Production and Plant Breeding, Faculty of Agriculture and Natural Resources, Imam Khomeini International University, Qazvin, Iran (phone: +989111297627; e-mail: R_khademian2005@yahoo.com).

Roghayeh Aminian, Assistant Professor, is with the Department of Production and Plant Breeding, Faculty of Agriculture and Natural Resources, Imam Khomeini International University, Qazvin, Iran (e-mail: Roghayehaminian@yahoo.com).

preventing the erosion especially on wet sandy soils or intercropping with crops of barley. In this study, the effects of different levels of salinity and calcium on the growth and morphological and some physiological properties of hairy vetch in a hydroponic system are studied [29].

II. Materials and Methods

A. Plant Material

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

B. Hydroponic Culture

In this study, a static culture system 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 photoperiod was 16h of light and 8h of darkness. The seeds are disinfected with sodium hypochlorite 1% for 15 mins, and then to germinate uniformly, seeds were planted in wet filter paper in Petri dishes, and were maintained in the growth chamber at 25 °C and 60% humidity. After germination and rootlet production, seedlings were transferred into holes of 5 × 5 cm on a floating polystyrene plastic containers measuring 18 × 27 × 37 cm when they were inside the tubes with net at their bottoms. Hoagland nutrient solution was used for the nutrition of seedlings, and after a week, salinity and calcium treatments were applied. Each pot contains 10 liters 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.

C. Treatments in Hydroponic System

A factorial experiment in a 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 [16], [17]. Three weeks after salinity stress, sampling of seedlings of hairy vetch was conducted to investigate the effects of stress on genotypes, and the following characteristics were measured.

D. Studied Characteristics

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 the accuracy of 0.0001.

Root and shoot dry weight: First, samples were put in an oven with temperature 72 °C for 48h and then were measured with a digital scale with the accuracy of 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 (four 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) determines the amount of chlorophyll as non-destructive [24].

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; one part was placed in a solution without the PEG and another part was in a solution with 40% PEG solution for 24h at 25 °C. After this period, the first EC reading was conducted. After the EC measurement, all leaves were maintained at the temperature of 120 °C and the pressure of 1 atmosphere, then they were autoclaved for 20 min. After the cooling of solutions (25 °C), the second readings were recorded. The membrane stability was calculated by:

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

where: T1 and T2 are the first readings of EC for treatment under stress with PEG and control, and C1 and C2 are the second readings for treatment under stress with PEG and control, respectively.

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

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

III. Results and Discussion

The results of variance analysis showed that there 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 ($P \leq 0.01$) and the relative water content of leaf and root length ($P \leq 0.05$). Two levels of calcium did not statistically show any significant difference for all measured indices. Calcium partly reduces the negative effects of stress but its effects were not statistically significant. Also, the interaction of the calcium and salinity was not significant for any measured parameters (Table I).

Comparison of the different levels of salinity showed that increasing the severity of salinity stress reduced the stem length, root length, shoot dry weight, root dry weight, leaf area, and biomass. Most characteristics belong to the control treatment, and these characteristics decreased with the increase of salinity so that the lowest values were observed in NaCl 100 mM. The most common adverse effect of salinity on most plants is the reduction of plant height and the quality of its products. Therefore, when the plant shoot growth has a severe decline under salinity stress, it damages the final performance of plant [27]. Disorder at plant growth and its loss under salinity stress are due to the decrease in photosynthetic level [4]. The previous studies have shown that salinity stress in wheat and barley causes to decrease the stems' length and the plant height [20].

Increasing the salinity also leads to reduce root length, so that the maximum root length of 37.81 mm and the minimum length 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 the root is directly confronted with stress [7]. Several studies have shown that salinity leads to decrease root length in tomatoes [14] and potatoes [13]. Other effect of salinity stress has been a decrease in stem length. The highest stem length of 41.5 mm and the lowest length 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, and similar results in [17] have also been obtained. Ion toxicity because of an increase in salinity leads to impair all biological and metabolic activities of plants, leading to an intense reduction or loss of shoot [9].

Results from the comparison of the mean values showed that an increase in the intensity of salinity stress also decreased plant leaf area, which can be due to the reduction of the absorption of water by the roots, and consequently reduction of water in the aerial parts of plant. The greatest leaf area with an average of 8.84 cm^3 and the lowest leaf area with an average of 4.28 cm^3 were observed in the control and 100 mM salinity treatments, respectively (Fig. 1).

TABLE I
ANALYSIS OF MEASURED CHARACTERISTICS

S.O.V	df	RWC	CMS	Chlorophyll (%)	leaf area (mm^2)	root length (mm)	shoot length (mm)	root dry weight (gr)	shoot dry length (gr)	biological yield (gr/m^2)
Salinity	2	*356.91	**1236.44	**115.25	**31.32	*118.06	**61.46	**216.50	**822.06	**1866.72
Calcium	1	0.12ns	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	1.73ns	18.86ns	4.00ns	0.64ns	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

*, ** And Ns: Respectively, Indicating A Significant Difference in the Level of 1%, 5% and No Significant Difference.

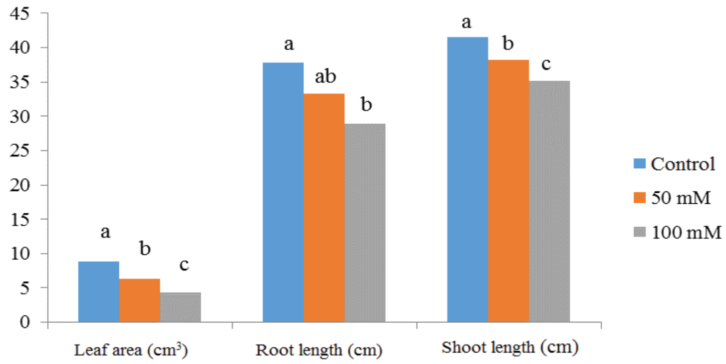

Fig. 1 The effect of different salinity levels on leaf area, root and stem length

The results showed that increasing salinity has decreased shoot dry weight, so that the maximum shoot dry weight (36.46 mg) and its lowest value (24.46 mg) were observed in the control and 100 mM salinity treatments, respectively. Adding calcium to the nutrient solution serves to reduce the negative effects resulting from salt stress, but this effect was not statistically significant (Fig. 2). This decline could be due to the large amounts of harmful ions such as Na+ and Cl- and disorder in the metabolism of the other nutrient elements caused by the ions [18].

The different levels of salinity had a significant effect on root dry weight, so that increasing the intensity of stress decreased the 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 mM salinity levels, respectively. Decreasing the root dry weight results in the negative effects of salinity stress, leading to the reduction of the production of roots and stems. The roots absorb water and minerals, and salinity stress arrives to the plants via roots. Therefore, the root is the first part that is confronted with stress. Plants consume much energy for making the organic materials such as proline, mannitol, glycine betaine and sorbitol as well as osmotic adjusting to deal with salinity. Thereby, the efficiency of roots decreases to provide nutrient elements and water for the other plant organs, causing to reduce the shoot growth. As a result, salinity stress decreases organogenesis, dry matter production, the transfer of nutrients from cotyledons to the embryonic axis, and finally the root and stem weight [11].

Biological yield is reduced because of the increasing 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 were observed (Fig. 2). The reduction of photosynthetic levels and the excessive consumption of energy to control and reduce the effect of salinity stress can be of the major factors of malfunction in many plants. Studies on wheat, barley, and lentil showed that increasing salinity stress decreases biological yield [17].

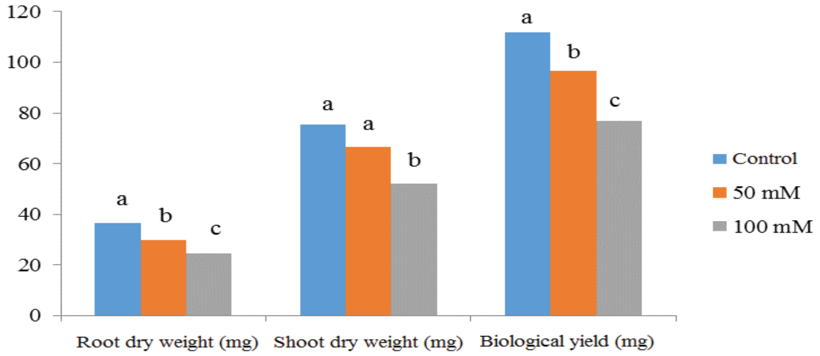

Fig. 2 The effect of different salinity levels on biological yield, shoot and root dried weight

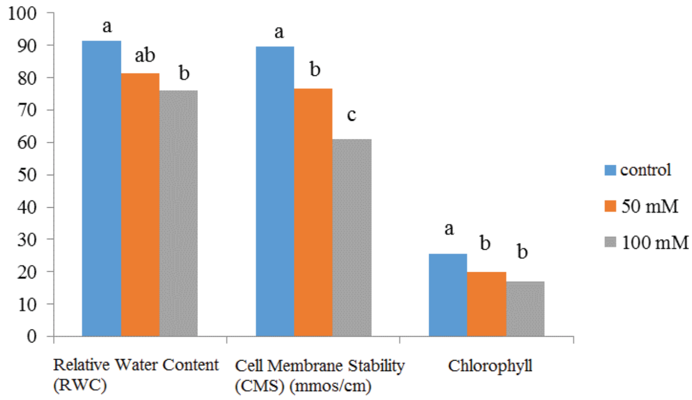

Fig. 3 The effect of different salinity levels on RWC, CMS and chlorophyll

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 by 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 have a great impact on the photosynthetic activity and dry matter production. A significant reduction of chlorophyll in the high concentrations of salinity stress results in the tissue destruction in chloroplasts [1]. It was reported that the destruction of chlorophyll in the high concentrations of salinity stress increases chlorophyll enzymes and minerals in chloroplasts, which would add ions to the chlorophylls [22]. In this test, relative water content on leaves measured decreased. The highest relative water content on leaves with an average of 91.3% at salinity stress level of zero and the lowest value with an average of 76.09% at salinity stress level of 100 mM were observed (Fig. 3).

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

The study of simple correlation among the characteristics showed that there is a highly significant correlation between them except the 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 the shoot dry weight and the relative water content on leaves was not significant (Table II). Among the properties, the plant biological yield had a high correlation with most characteristics, and the highest amount was related to the correlation of this property with the shoot dry weight (r=0.96). As mentioned above, the root length has a little correlation with the other properties, and the lowest value has been associated with the amount of chlorophyll on leaves (r=0.30). Also, [2] reported that chlorophyll and membrane stability index had a positive and significant correlation with the yield. Identifying physiological characteristics affecting the yield has been mentioned as one of the most important ways to modify the yield.

TABLE II
REGRESSION OF STUDIED CHARACTERISTICS

Characteristics	RWC	CMS	Chlorophyll (%)	Leaf area (mm^2)	Root length (mm)	Shoot length (mm)	Root dry weight (gr)	Shoot dry weight (gr)	Biological yield (gr/m^2)
RWC	1								
CMS	**0.666	1							
Chlorophyll	*0.525	*0.560	1						
Leaf area	*0.461	**0.714	*0.527	1					
Root length	0.085^{ns}	0.360^{ns}	0.305^{ns}	0.459^{ns}	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	0.382^{ns}	**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

*, ** And Ns: Respectively, Indicating A Significant Difference in the Level of 1%, 5% and No Significant Difference.

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