Effect of salinity on seed germination , growth and organic compounds of mungbean plant *Vigna radiata* (L.) Wilczek .

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Abstract

Laboratory experiment in Petri dishes was carried out to investigate the effect of different salt concentration levels (0 , 50 , 100 , 150 and 200) mMol / L of sodium chloride on the seeds germination and growth of mung bean plant . The results of the study showed that , the increase in salinity concentration caused a decrease in seeds germination percentages (%97 , %96 , %95 and %82) respectively as compared with germination percentage (%100) with a control treatment ; the stem lengths , fresh and dry matter weights decreased as a result of the increase of salinity at all the treatments when salinity level increased . Another experiment in plastic pots was adapted to study the effect of the salinity at the same concentration levels on the carbohydrates , proteins and proline concentrations . The results showed that the increase of salinity caused a decrease in the carbohydrate content , whereas the high content (33) µg / gm of carbohydrates was observed with the control treatment , whereas the low content of carbohydrates (15) µg / gm found with the salt treatment (200) mMol / L . The effect of salinity on the proteins and proline occurred at the inverse of carbohydrates , it caused a gradual increase on their contents , and the high contents (141 and 14) µg / gm respectively were observed at the salt treatment (200) mMol / L . A significant differences on the contents of proteins and proline between the salt treatments were found .

تأثير الملوحة على إنبات البذور والنمو والمركبات العضوية في نبات الماش *Vigna radiata* (L.) Wilczek .

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المستخلص

أجريت دراسة مختبريه في أطباق بتري لمعرفة تأثير مستويات تراكيز ملحية مختلفة (٠ و ٥٠ و ١٠٠ و ١٥٠ و ٢٠٠) ملي مول / لتر من كلوريد الصوديوم ، على إنبات البذور والنمو لنبات الماش . أظهرت نتائج الدراسة إن زيادة الملوحة سببت خفضاً في نسب إنبات البذور(٩٧% و ٩٦% و ٩٥% و ٨٢%) ، على التوالي بالمقارنة مع نسبة الإنبات (١٠٠%) عند معاملة السيطرة . أما النمو وكما هو مقاس في أطوال السيقان والأوزان الرطبة والجافة لها ، فقد إنخفض كنتيجة لزيادة الملوحة وفي المعاملات الملحية

كافة . وأعدت تجربة أخرى في سنادين بلاستيكية لدراسة تأثير الملح وبمستويات التراكيز نفسها على محتوى الكاربوهيدرات والبروتينات والبرولين . أظهرت نتائج الدراسة إن زيادة الملوحة سببت خفضاً في محتوى الكاربوهيدرات ، و لوحظ المحتوى المرتفع منها (٣٣) مايكرو غرام / غم في معاملة السيطرة ، بينما ظهر المحتوى المنخفض (١٥) مايكرو غرام / غم في المعاملة الملحية (٢٠٠) ملي مول / لتر . في حين كان تأثير الملوحة على البروتينات والبرولين على العكس من تأثيرها في الكاربوهيدرات ، فسببت زيادة تدريجية في محتواهما ، وظهرت المحتويات المرتفعة منهما (١٤١ و ١٤) مايكرو غرام / غم في المعاملة الملحية (٢٠٠) ملي مول / لتر ، وقد وجدت فروق معنوية في محتوى البروتينات والبرولين بين المعاملات الملحية .

Introduction

Soil salinity is a major concern to the agriculture in arid and semi - arid regions . According to an estimation one - third of the worlds land surface is arid or semi - arid (4.8×10^9 ha.) , out of which one - half is estimated to be affected by salinity (Bradbury and Ahmad , 1990) . The problems of salinization are increasing , either due to bad irrigation drainage or agriculture practices . Despite its relatively small area , irrigated land is estimated to produce one - third of the world food (Munns , 2002) . Mung is widely grown in the south regions of Iraq where salinity is a common problem . The problem of soil salinity which particularly appears in the course of irrigation , leading from seriously diminished yield to a complete loss of land suitability , has a major importance in many areas with arid and semi - arid climatic conditions (Doering *et al.*, 1984) . The vast area of land is becoming unproductive each year due to ever - increasing salt accumulation . Salinity Stress causes an imbalance in the uptake of mineral nutrients and their distribution within the plants (Grattan and Grieve , 1992 ; Glenn *et al.*, 1999) . Under salinity conditions depression of germination percentages is usually takes place by a combined effect of seed imbibitions capacity as a result of low osmotic potential of the soil solutions (Dutt , 1976) and specific ion effect (Hassen , 1999) . Increasing concentration of salts reduced the seed germination percentages and growth of many crops were reported by many authors (Kassray and Doering , 1989 ; Al-Zubaydi *et al.* , 1992 ; Nasir 2002 ; Al - Seedi , 2004). The metabolic response of the plants to the salt stress was the constitution of a compatible osmotic compounds , which played an important role to conservation the inner cellular structures , and decreased the harmful of oxidation (Rhodes and Hanson , 1993) .The compatible osmotic compound includes carbohydrates , amino acids , ammonium compounds and polyols . Carbohydrates executes an important role on the osmoregulation of plant cells , especially their plants , which are growing under the effect of salt stress (Gorham *et al.*, 1981).The incurrence of plants to salt stress leads to the accumulation of some organic solutes as sugar , proline , betaine , amino acid , proteins and carbohydrates which are importance on the osmoregulation (Gorham *et al.*, 1981 ; Bolarin *et al.*, 1995 ; Serrano *et al.*, 1999 ; Saffan , 2008) .The present investigation was undertaken to study the effect of salinity on germination , growth and the organic compounds content of mungbean plant .

Materials and Methods

1 -*First experiment*

Seeds of mungbean (local variety) were surface sterilized with (%6) sodium hypo-chlorate solution for (10) minutes and then washed with distill water before utilization , the seeds were sowed in the experiment. Salt solution was prepared to give the concentrations levels (50, 100, 150 and 200) m Mol/L. of NaCl , in addition to distill water was applied as a control treatment. Twenty five seeds were germinated in four Petri dishes (10 cm. diam.) and replicated three times . Two filter paper were put in each dish and moistened with (7) ml. of salt solution and incubated at 25C°. After (3) days germinated seeds were recorded and the percentage of germination was calculated . The length in (cm) of seedlings epicotyls were measured after (7) days at the beginning of germination and the fresh weights were calculated . The fresh matter was dried in an oven at (65) C for (48) hours to determine the dry weights of shoots .

2 - *Second experiment*

The experiment was carried out on Dept. of biology , College of education , Thi -Qar Univ . by using a plastic pots (15 cm. diam.) containing (1) kg. of sandy-loam soil with EC (3.6) dS/m and pH (7.23) . Treatments were replicated three times .Salt solution was prepared to give the same concentration levels , and was added as an irrigation water to plastic pots , in addition to distill water treatment was applied as a control. Ten seeds had chosen and sowed in each pot at a depth (1) cm . At the beginning of the experiment , pots were irrigated by distill water up to soil field capacity until the seedling reached (7) days old . These plant seedlings had received the salt solution for a period of (14) days . After the plants were harvested and washed with distill water , some of them were prepared to the estimation of carbohydrates and proteins whereas the others dried in an oven at (65) C for (48) hours. The dry matter of the plant shoot systems was weighted and used for the estimation of proline.

A- *The estimation of carbohydrates and proteins* :

For estimation of the soluble carbohydrates and proteins , (200) mg of fresh weight of the plants were taken and crushed with (10) ml. of distill water , then the solution centrifuged for (15) minutes and the clear solution was heated in water bath at (50) C for (30) minutes . The centrifuge process was repeated again for (15) minutes and then the absorbance of the clear solution was measured by spectrophotometer (Spectro SC Labomed Inc. U.S.A.) at the wave length (490) nm. for carbohydrates and (600) nm. for proteins . The total carbohydrates and protein were estimated according to the procedure described by (Herbert *et al*., 1971) .

B – *The estimation of proline* :

1 - Preparation of proline standard calibration curve

It was prepared from different concentration ranged from (1 - 10) mg / L. by using the pure proline (100) mg that dissolved in one liter of a distill water .

From each concentration (3) ml. had taken and putting in a glass test tube , (3) ml. of a glacial acetic acid and (5) ml. of ninhydrin acid were added and mixed . The mixture was heated in a water bath at boiling point for (30) minutes , then elicit and let it cool . Toluene (5)ml. was added to a mixture and let it for an appropriate period of time , then the optical density for the different concentrations of pure proline (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10) mg / L. were measured at the wave length (520) nm. and the results were recorded .

2 - The estimation of proline in plant tissues

The green tissue of the plants was washed with distill water and air dried at a temperature of laboratory (25 - 30) C. The dry matter was ground with an electric blender , (100) mg of the ground matter had taken and put

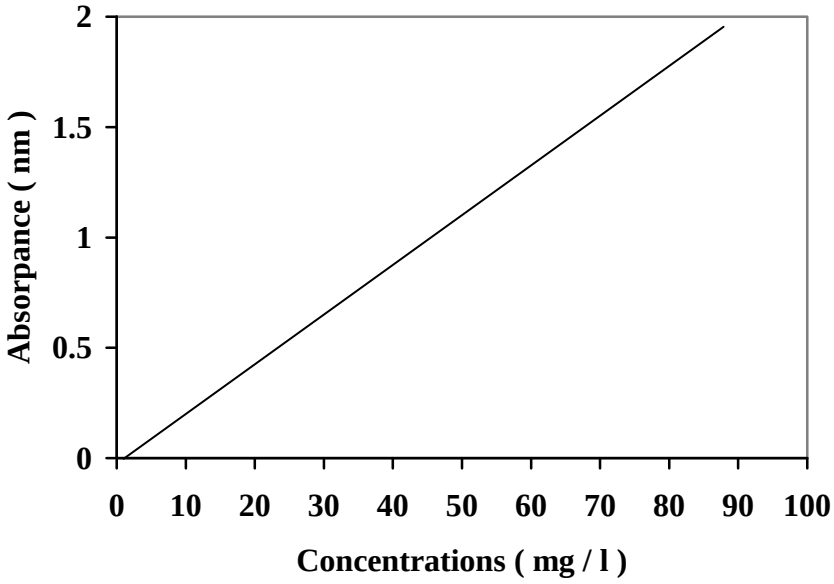


Diagram (1) proline standard calibration curve

into a glass cup , (5) ml. of sulfosalsalic acid (%3) was added and crushed well with hand tool . The mixture was put in a marked glass test tubes and centrifuged by the centrifuge (Fanem Excelsa II Mod. 206 BL.) at (3300) circle for (5) minutes , after the clear abstract was infused to a new glass test tubes and mixed with (3) ml. of a glacial acetic acid and (3)ml. of ninhydrin acid . The glass test tubes are put in a water bath (TLI- Thermo lab .IND.) at boiling point for (30) minutes . After all the tubes were elicited and cooled , the red colour noticed , it was due to the reaction of proline with ninhydrin acid which was separated by adding (5) ml. of toluene . The red toluene layer was measured by spectrophotometer at the wave length (520) nm. according to the procedure described by (Bates *et al.*, 1973) . The obtained data were subjected to statistical analysis of variance and the T. test values at (P < 0.05) level of significance were calculated .Test of significance was done according to the least significant differences (L.S.D) for each salt treatment by using the statistical program (Spss - 11- 2003) .

Results and Discussion

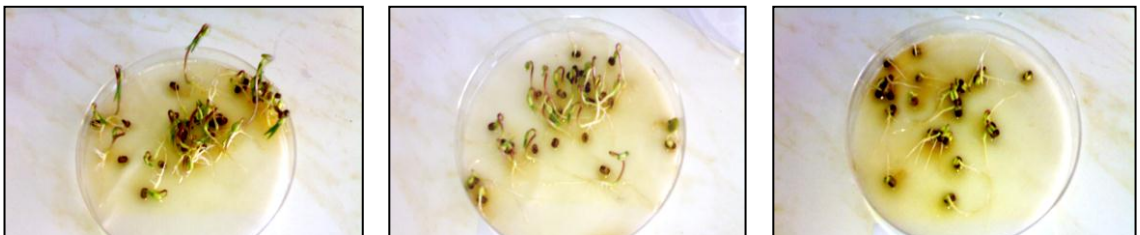
Table (1) Show that the high percentage of germination (%100) was occurred in distill water at a control treatment , whereas a gradual decrease on the percentages of germination were noticed with the increase of salt concentrations at the all treatments . The high percentage of germination (%97) was observed at the salt treatment (50) mMol / L. , whereas the low percentage (%82) occurred at the treatment (200) mMol / L.

Table (1) The effect of salinity on the percentages of germination , lengths of seedlings and their fresh and dry matter weights (mean of 5 plants)

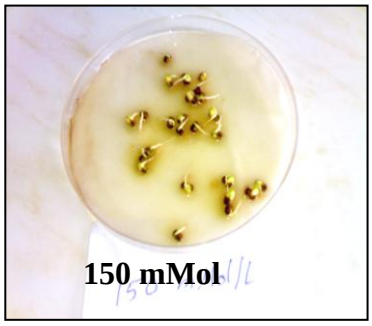
Salt treatments m Mol / L	germination Percentages (%)	Lengths of seedlings (cm.)	Fresh weights (mg)	Dry matter weights (mg)
Control	a 100	a 6.60	a 665	a 128
50	ab 97	a 5.80	b 545	b 89
100	b 96	b 4.50	b 473	b 81
150	b 95	c 3.20	c 329	c 59
200	c 82	d 1.44	d 174	d 27
L.S.D	3.40	1.034	98.20	20.60

***Numbers having the same alphabetical letters are not different at (0.05) level of significance .*

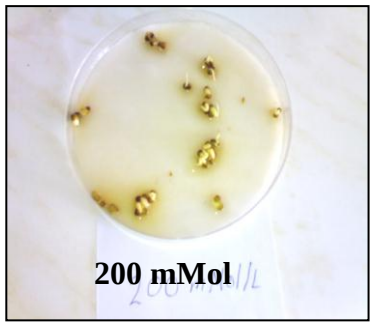
A significant differences on the percentages of germination **between a control treatment and the salt treatments (100 , 150 and 200) mMol / L. were noticed .The reduction of the percentages of germination with increasing salinity was due to the specific ion effect (Hassen , 1999) or to the limited water supply as a result of low osmotic potential (Dutt , 1976). The negative effect of salinity during germination was due to the toxic and**



Control **50 mMol** **100 mMol**



150 mMol



200 mMol

All treatments

**** Pictures are illustrates the effect of salinity on the germination of seeds of mung bean plant after (48) hours of the beginning process of germination .***

osmotic effects of salt ions especially sodium and chloride (Khan *et al.*, 1999 ; Tester and Davenport , 2003) . These results are in accordance with many authors (Al - Zubaydi *et al.* , 1992 ; Nasir , 2002 ; Al-Seedi , 2004) .

From the previous table it was observed that , salinity affected lengths , fresh and dry matter weights of seedlings . A significant differences between the all treatments were noticed . The high values of means of lengths and weights occurred on the distill water at a control treatment , whereas the low values of means occurred at the high salt treatment of (200) mMol / L . A gradual decrease with increasing salt concentrations levels was noticed . The increase of salinity on the plant growth medium caused a reduction in plant selective ability to absorb the other important ions for the growth especially potassium , that was resulted from a toxic accumulation of sodium ions on the plant tissues (Torres , 1972) . Also the accumulation of toxic ions within plants caused a nutritional imbalance that leads to the reduction of growth (Al-Zubaydi and Al-Seedi , 1999) .

The reduction of plant growth under salinity was due to the effect of salinity on the different vital activities of plants , such as a depression on the enzymes activities , metabolism , cells division and photosynthesis (Mayer *et al.*, 1973) . These results are in accordance with many authors (Al - Zubaydi *et al.* , 1992 ; Hassen , 1999 and Al - Seedi , 2004).

Table (2) demonstrated the effect of salinity on the organic compound of mung bean plants. It was a clear that salinity affected the soluble carbohydrates and a gradual decrease was noticed with increasing concentrations of salinity. The

high concentration of carbohydrates (33) µg / gm occurred in the distill water at a control treatment , whereas the low concentration (15) µg / gm was observed at the treatment of (200) mMol / L . A significant differences between a control and the other treatments occurred . The plants , grow in a saline environment suffered from the increase of the osmotic stress as a result of the increase of the concentration of salt ions , which leads to the entrance of these ions and their concentration increased in the cells sap of tissues , which causes a negative effect on the plant growth (Alfocea and Bolarin , 1996). The increase of salt ion concentrations , especially sodium and chloride on the growth medium of plants causes an imbalance on the osmotic potential , and leads to a decrease of the essential mineral nutrients , that affected the synthesis processes and the plant growth (Prakash and Widholm , 1993). The depression of plant growth was due to salinity , that high salinity caused a decrease on the assimilation of CO_2 throw the effect on the opening of stomata and the sufficiency of photosynthesis process (Ungar , 1991) . Also , high salinity affected the stroma volumes of the chloroplasts , and arises of a reactive oxygen species (ROS) which played an important role on the depression of the sufficiency photosynthesis process of the plants (Price and Hendry , 1991) . The depression of plant growth was due to the

Table (2) the effect of salinity on the organic compounds of the mung bean plants .

Salt treatments m Mol / L	Carbohydrates µg / gm (Fresh weight)	Proteins µg / gm (Fresh weight)	Proline µg / gm (Dry weight)
Control	a 33	c 82	d 6.0
50	b 28	bc 92	c 8.0
100	b 26	b 100	c 9.0
150	c 17	a 133	b 11.0
200	c 15	a 141	a 14.0
L.S.D	3.60	11.80	1.60

effect of salinity on the protein bonds of green pigments . It was found that the adverse relationship between salinity and growth , that high salinity affected the protein bonds of green pigments and caused a cute decrease on the chlorophyll content (Rivera and Heras , 1973) .

The increasing of salinity concentration caused an inhibition on the enzymes formation that participates on the chlorophyll molecules formation , such as a chlorophyllase which participated in the formation of chlorophyllids and phytol (Sivtsev *et al*., 1973) .

These reasons actually explained the depression on the plant growth and the carbohydrate concentrations with increasing concentration levels of salinity . From the table above , it was observed that salinity causes an increase on proteins and proline of the plant tissues , a gradual increase occurred with increasing concentration levels of salinity . The low concentrations of protein and proline (82 and 6.0) µg / gm occurred on the distill water at a control treatment respectively , while the high concentrations (141 and 14.0) µg / gm were noticed at the treatment (200) mMol / L . A significant differences between salinity treatments were obtained of protein and proline concentration .

The response of plants to the osmotic stress was based on the construction process on the number of the defense proteins, the plants defense against the affect of salinity by the osmoregulation process which continues by the biosynthesis of the solutes on the cells (Serrano *et al.,* 1999) . Increasing salinity concentration levels leads to the an increase on the absorbance of some essential elements that activated the action of some enzymes , which were essential for the protein synthesis (Rakova *et al.,* 1969). The incurrence of plants to the salt stress leads to the accumulation of some organic solutes such as sugar , betaine and proline , these compounds are important for plants on the osmoregulation (Gorham *et al.,*1981) .The increase of the proline concentration on the tissues of plants that grow in a saline environment was resulted from the imbalance on the osmoregulation inside the cells , that was due to the increases of salts on the growth medium . The increases of proline concentrate on the cells to a creation the case of osmotic balance inside the cells especially between vacuoles and the cytoplasm (Naidu , 2003) .

The important properties of salinity was the affectation of the dominance of protein and some of amino acids , especially proline on the plants . These organic compounds increased according to the increase of salinity (Abo-Zaid , 2000) . The obtained results are in accordance with many authors (Rhodes and Hanson , 1993 ; Bolarin *et al.,* 1995 ; Saffan , 2008) .

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