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EFFECTS OF SALT STRESS ON CARBOHYDRATES, ORGANIC ACIDS AND AMINO ACID COMPOSITION OF SALT-SENSITIVE AND SALT-TOLERANT LETTUCE CULTIVARS

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ABSTRACT

Salinization of both salt-sensitive and salt-tolerant lettuce plants with increasing doses of NaCl, throughout the entire period of experiment induced significant increases in both glucose and fructose contents and significant decreases in sucrose content. In salt-tolerant Eskandrany cultivar, sucrose content was found either to increase significantly (at 3 dS/m NaCl), non-significant decrease (at 7 dS/m NaCl) or to decrease significantly (9 dS/m NaCl). Organic acid content (citric, oxalic and keto acids) showed significant increases in both salt-sensitive and salt-tolerant lettuce cultivars as compared with control. Proline and glycine amino acids content of both cultivars, salinized with increasing doses of NaCl, throughout the entire period of the experiment, showed significant increases. In all cases, the magnitude of accumulation of all metabolites determined was most pronounced in salt-tolerant lettuce cultivar. The results were discussed in relation to the mechanisms of salt tolerance in plants.

Keywords: Lettuce, NaCl, carbohydrates, amino acids, organic acids.

INTRODUCTION

Plant response to salinity is generally described in terms of relative yield as a continuous function of root zone salinity, expressed as electrical conductivity of the solution in contact with the roots (ECe) (Maas and Hoffman, 1977, Maggio *et al*., 2007). Tammam *et al*. (2008) demonstrated a remarkable variation in accumulation of different carbohydrate fractions among wheat studied organs. Salt stress has a different effect on carbohydrate contents. Some authors have reported carbohydrates accumulation in various plants under salinity condition (Parida *et al*., 2003 and Azooz *et al*., 2004). Mostafa (2004) observed that at low and moderate salinity levels, sugars and consequently the total carbohydrates are decreased. Many plants accumulate compatible osmolytes, such as proline, Gly betaine, or sugar alcohols, when they are subjected to osmotic stress (Delauney and Verma, 1993; Satoh *et al*., 2002). Among these, proline is the most diversely used osmolyte accumulated under osmotic stress conditions in plants (Delauney and Verma, 1993; Satoh *et al*., 2002). Amino acids have been reported to accumulate in higher plants under salinity stress (Ashraf, 1994; Mansour, 2000). The important amino acids include alanine, arginine, glycine, serine, leucine, and valine, together with the imino acid, proline, and the non-protein amino acids, citrulline and ornithine (Rabe, 1990; Mansour, 2000). An increase in citrate, malate, and lactate and a decrease in glycollate and glycerate have also been observed in *V. faba* roots after withholding of water (Venekamp *et al*., 1989; Fougere *et al*., 1991). In alfalfa roots and nodules subjected to salt stress, organic acids did not contribute to the restoration of the osmotic potential and osmotic adjustment, but lactate

should be given specific attention, because its concentration was doubled after salt stress. The objectives of this study were to study, the effects of NaCl on carbohydrate, organic acids and amino acids contents in salt-sensitive and salt tolerant lettuce plants.

MATERIALS AND METHODS

Plant material

Transplants (35-d-old) of two cultivars of *Lactuca sativa*; salt-tolerant cultivar (Eskandrany) and salt-sensitive cultivar (Baladi) were kindly supplied by the Horticulture Research Centre, Ministry of Agriculture, Giza, Egypt.

Estimation of carbohydrates

The method of extraction of different soluble sugars used in this investigation was patterned after those adopted by Yemm and Willis (1954) and Handel (1968). Glucose was estimated in the ethanolic extract of lettuce using the o-toluidine procedure of Fertris (1965). Fructose was estimated in the lettuce ethanolic extract using the resorcinol method of Roe (1934). Sucrose content was determined using the procedures adopted by Yemm and Willis (1954) and Handel (1968). Sucrose content was determined by first degrading reactive sucrose present in 0.1 cm^3 of the ethanolic extract with 0.1 cm^3 5.4 N KOH at 97 ^0C for 10 min. Three cm^3 of freshly prepared anthrone reagent [150 mg anthrone + 100 cm^3 72% (w/w) H_2SO_4] were then added to the cooled reaction products and the mixture was heated at 97 ^0C for 5 min, cooled and the developed colour was read at 630 nm, using spectrophotometer.

Estimation of amino acids

The method used in this investigation was essentially that adopted by Yemm and Willis (1956) as described by Hasaneen (1978). Glycine was determined using the method of Muting and Kaiser (1963). Proline was estimated using the method of Snell and Snell (1954).

Estimation of organic acids

The methods adopted for estimation of oxalic acid and citric acid were essentially those of Snell and Snell (1949). Keto acids were estimated by the method of Freidman and Haugen (1943).

Time course experiment

Homogenous transplants of lettuce (*lactuca sativa* L. cv. Baladi and lactuca sativa L. cv. Eskandrany) were used. The details of the experimental setup as well as of essentially those described by El-Bialy (2005). The transplant (35-d-old) were washed thoroughly with tap water and then transplanted in a mixture of clay-loamy soil (2:1, v/v)in pots (30× 28×26 cm) all pots contained equal amounts of homogenous soil (8 kg). The experiments were carried out outdoor under normal day and light conditions. In all cases, treatment of lettuce transplants with NaCl was carried out after one week from the date of transplantation. All pots were irrigated with tap water, every three days, to maintain the soil at the field capacity throughout the experiment. The appropriate amounts of NaCl were calculated and recorded with irrigation water so as to maintain salinity levels at 3 dS/m, 7 dS/m and 9 dS/m. For control as well as for the differently treated plants, superphosphate was applied with irrigation water as 0.8 g pot^{-1} every three

weeks. Samples transplantation representing the vegetative, flowering, fruiting and yield growth stages were taken after 53, 104, 155 and 177 days from the date of transplantation, respectively. The samples were used for determination of carbohydrate fractions, organic acids and amino acids fractions contents. Experimental data were subjected to one way analysis of variance (ANOVA) with post-hoc LSD (Least Significant Difference) test. A P value < 0.05 was accepted statistically significant. Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 13.0, 2004, Chicago, IL, USA).

RESULTS AND DISCUSSION

Changes in carbohydrate content

Perusal of the data illustrated graphically in fig. 1 and 2, revealed that salt-sensitive and salt-tolerant lettuce cultivar salinized with low, moderate and high level of NaCl showed variable significant increases in both glucose and fructose contents and variable significant decreases in sucrose content, throughout the entire period of experiment. In salt-tolerant skandarany cultivar, sucrose content was found either to increase significantly (at 3 dS/m NaCl), non-significant decrease (at 7 dS/m NaCl) or to decrease significantly (at 9 dS/m NaCl).

In support of these results, carbohydrate changes are of particular importance because of their direct relationship with such physiological processes as photosynthesis, translocation, and respiration. Among the soluble carbohydrates, sucrose and fructans have a potential role in adaptation to these stresses (McKersie and Leshem, 1994; Kerepesi and Galiba, 2000).

A tentative explaination of the change in carbohydrate fractions in in salt-sensitive and salt-tolerant lettuce cultivars salinized with increasing levels of salinity throughout the entire period of the experiment, in the present study was that sucrose can act in water replacement to maintain membrane phospholipids in the liquid-crystalline phase and to prevent structural changes in soluble proteins. The role of reducing sugars (glucose and fructose) in the adaptive mechanism is more controversial, and even their accumulation can be detrimental from several points of view (Kerepesi and Galiba, 2000).

Changes in organic acid content

The retained from this study are quite clear that the salt tolerance of *lactuca sativa* induced significant variable accumulation of organic acids and total phenolic compound contents, throughout the entire period of the experiment (see figure 3 and 4). In general, the accumulation rate of both organic acids and total phenolic compounds in the salinized lettuce plants, throughout the entire period of the experiment showed drastic increase in salt-tolerant cultivar compared with salt-sensitive cultivar.

The observed increases in organic acids (citric, oxalic and keto acids) contents as well as in total phenolic compounds in salt-

sensitive and salt-tolerant lettuce cultivars, throughout the entire period of the experiment, can be used as indicator in the selection of plants that will withstand saline stress through osmoregulations as reported by El-Saht (1994). Of interest in this connection, Handa *et al.* (1983) and Hasaneen *et al.* (1990) found that the concentrations of amino acids determined were increased with the degree of adaptation of tomato, French bean and maize plants to salt stress. Thus, the low tolerant French bean plants were found to accumulate less organic acids than the highly tolerant plants of maize.

El-Saht (1995) reported that keto acids, oxalic acid and citric acid contents of maize plants subjected to 100 mM NaCl showed highly significant accumulation of these acids. Accumulation of osmolytes allows osmoregulation to occur and may also stabilize macromolecules.

Changes in amino acids content

Perusal of the data presented and figure 5 and 6 revealed that proline and glycine contents of salt-sensitive and salt-tolerant lettuce plants treated with increasing concentration NaCl showed a significant increases, throughout the entire period of the experiment. At the highest level (9 dS/m) NaCl, the proline percent of production in salt sensitive cultivars 151.90 % at vegetative and 122.36% at flowering, 125.36% at fruiting and 132.54% at yield where in salt-tolerant cultivars the rate of accumulation of proline was 29.83% at vegetative and 22.65% at flowering, 20.21% at fruiting and 25.04% at yield stages. On the other hand, the accumulation rate of glycine in salt-sensitive cultivar was 127.05% at vegetative and 70.99% at flowering, 140.64% at fruiting and 86.46% at yield where in salt-tolerant cultivar the rate of accumulation of glycine was 123.23% at vegetative and 98.99% at flowering , 100.59% at fruiting and 75.23% at yield stages.

Free amino acids (proline and glycine) are known to occur widely in higher plants and normally accumulate in large quantities in response to environmental stresses (Hsu *et al.*, 2003; Kavi Kishore *et al.*, 2005; Ashraf and Foolad, 2007). In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures (e.g. membranes and proteins), scavenging free radicals, and buffering cellular redox potential under stress conditions. It may also function as a proteincompatible hydrotrope (Srinivas and Balasubramanian, 1995), alleviating cytoplasmic acidosis, and maintaining appropriate NADP+/NADPH ratios compatible with metabolism (Hare and Cress, 1997). Hasaneen *et al*. (2008) reported that salinization of lettuce plants with increasing concentration of NaCl induced significant increases in both proline and glycine contents which appeared to be progressively higher with an increase in salinity level used. In addition, increased proline and glycine contents in both salt-sensitive and salt-tolerant lettuce cultivars salinized with increasing concentration of NaCl, throughout the entire period of the experiment is started to be possibly related with NaCl tolerance (Juncklang, 2005). He added, higher accumulation of amino acids, glycine and proline contents were observed with increasing NaCl concentrations in both plant species.

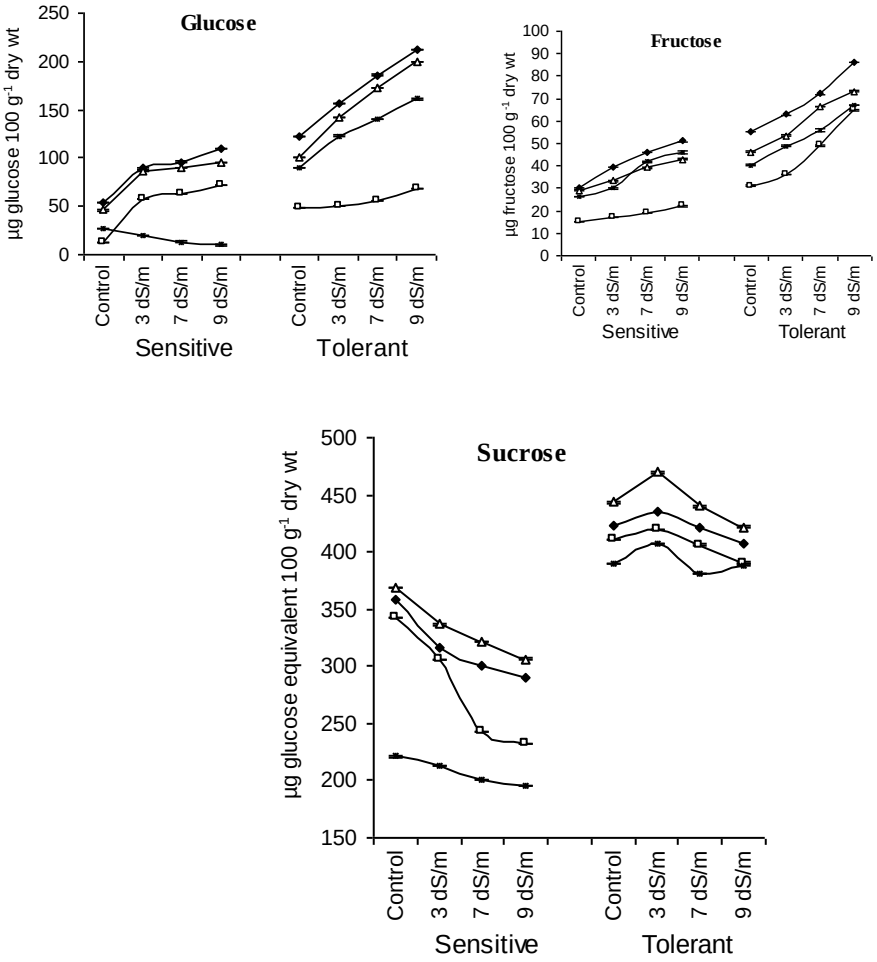

Fig. 1: The effects of three different levels of NaCl on soluble sugars (glucose, fructose, sucrose) of both salt-sensitive and salt-tolerant lettuce cultivars at four different stages (—□— vegetative, —♦— flowering, —Δ— fruiting, —*— yield). Vertical bars represent standard error (n=8).

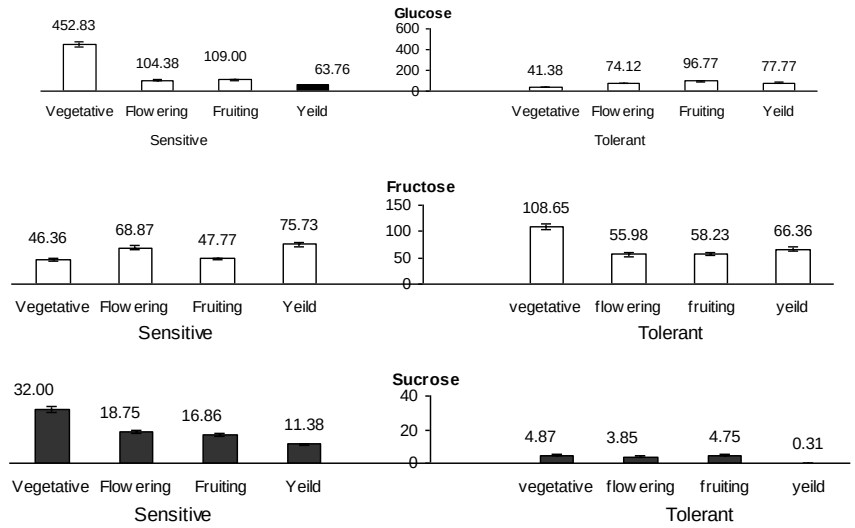

Fig. 2: Percent stimulation (PS) of both the glucose and fructose contents and percent inhibition (PI) of sucrose content of both salt-sensitive and salt-tolerant cultivars salinized with 9 dS/m NaCl at vegetative, flowering, fruiting and yield stages (□ PS, ■ PI). Vertical bars represent standard error (n=8).

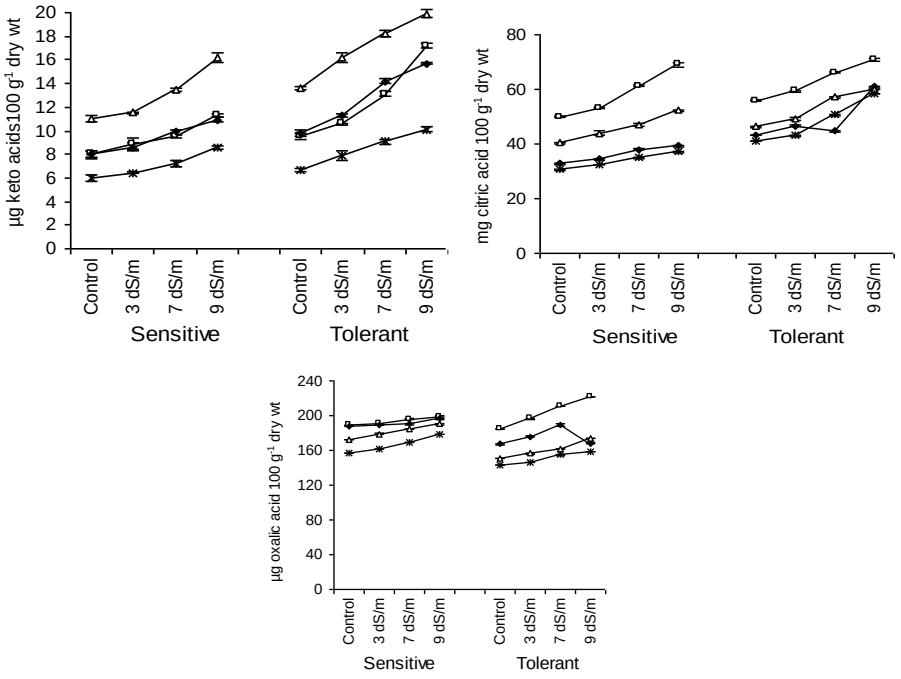

Fig. 3: The effects of three different levels of NaCl on organic acids (citric, oxalic and keto acids) of both salt-sensitive and salt-tolerant lettuce plants at four different stages (—□— vegetative, —♦— flowering, —Δ— fruiting, — * — yield). Vertical bars represent standard error (n=8).

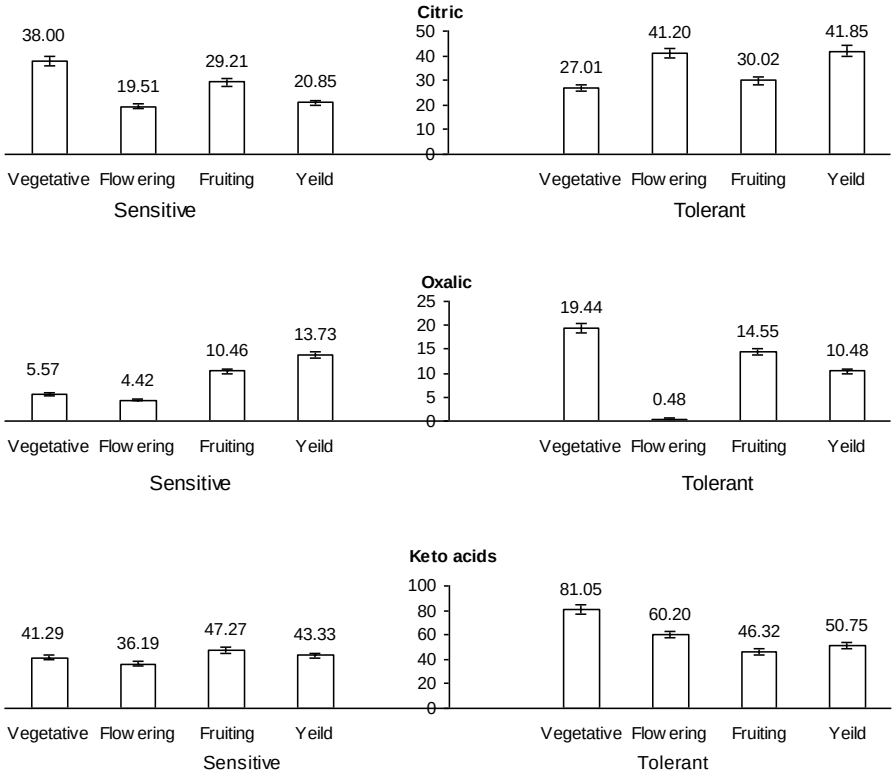

Fig. 4: Percent stimulation (PS) of organic acids of both salt-sensitive and salt-tolerant cultivars salinized with 9 dS/m NaCl at vegetative, flowering, fruiting and yield stages. Vertical bars represent standard error (n=8).

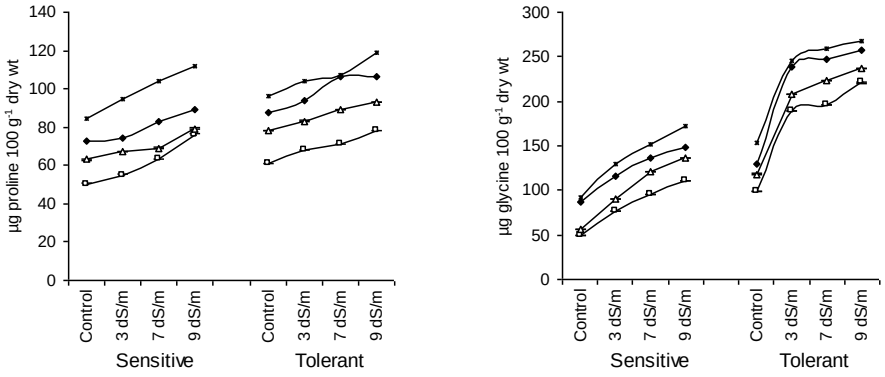

Fig. 5: The effects of three different levels of NaCl on amino acids (proline, glycine) contents of both salt-sensitive and salt-tolerant lettuce cultivars at different stages (—□— vegetative, —♦— flowering, —Δ— fruiting, — * — yield). Vertical bars represent standard error (n=8).

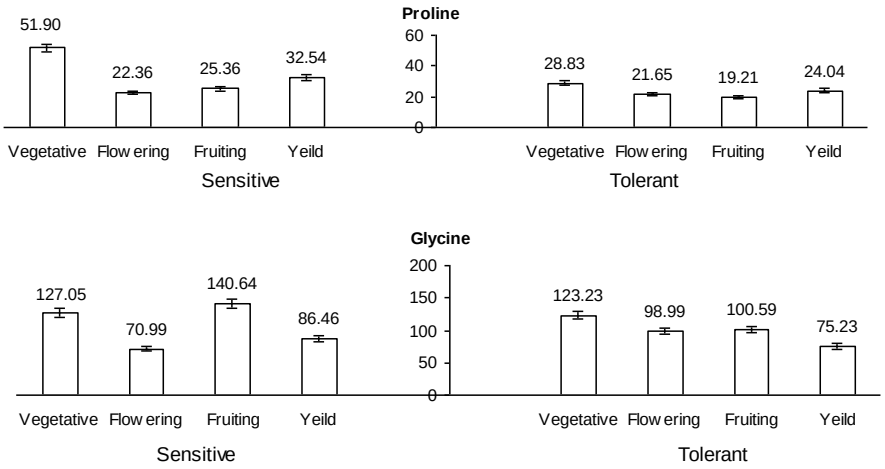

Fig. 6: Percent stimulation (PS) of amino acids of both salt-sensitive and salt-tolerant cultivars salinized with 9dS/m NaCl at different stages. Vertical bars represent standard error (n=8).

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"تـأثير الإجهـاد الملحـي علـي المحتـوي الكربوهيـدرات والأحمـاض العضـوية والأحماض الأمينية في صنف نبات الخس الحساس والمقاوم للملوحة"

محمود الباز يونس ، محمد نجيب عبدالغني حسنين و داليا محمد عادل مصطفي البيلي

قسم النبات – كلية العلوم – جامعة المنصورة – المنصورة - مصر

تم في هذا البحث زراعة شتلات صنفين من نبات الخس احدها حساس والإخر مقاوم للملوحه في خليط من تربه طينيه وطفليه بنسبة ١:٢ أما خاليه (كعينات ضابطه) أو مضاف اليها أي من مستويات ثلاثه من ملح كلوريد الصوديوم (٣، ٧، ٩ ديسي سيمنز/متر). ولقد صممت هذه التجربه بهدف دراسة تأثر صنف الخس الحساس والمقاوم للملوحه بالمستويات المتدرجة من الملوحه و اثر ذلك علي التغيرات الأيضيه في كل من المواد الكربوهيدراتيه (جلوكوز – فركتوز – سكروز)، الأحماض العضويه (الأوكساليك – الستريك –الأحماض الكيتونيه) والأحماض الأمنيه (حمض الجليسين وحمض البرولين) وذلك اثناء مراحل النمو والتطور المختلفه (النمو الخضري – الأزهار – الأثمار – الحصاد).بالمقارنه بالنباتات غير المجهده في صنف الخس – لوحظ ان معاملة صنف الخس بالتركيزات المتدرجه من ملح كلوريد الصوديوم و أثناء فترات النمو والتطور المختلفه – أدت الي حدوث زيادات ممعنويه في محتوها من الجلوكوز والفركتوز اما سكر السكروز فلقد لوحظ انه يزداد في حالة معاملة النباتات المقاومه بتركيز ٣ ديسي سيمنز/متر وينقص بدرجه غير معنويه عند المعامله بتركيز ٧ ديسي سيمنز/متر و ينقص معنويا عند المعامله بتركيز ٩ ديسي سيمنز/متر. أما الأحماض العضويه في كلا الصنفين فلقد لوحظ أن المعامله بالتركيزات المتدرجه من الملح ادت الي زيادات معنويه في محتواهم في كلا الصنفين خلال فترات النمو والتطور كذلك لوحظ أن محتوي الصنفين من الأحماض الأمنيه (حمض الجليسين –حمض البرولين) قد ازداد زياده معنويه كبيرة عند معاملةالصنفين بالتركيزات المتدرجه من الملح وخلال فترات النمو والتطور المختلفه وبصفه عامه فلقد لوحظ أن الزيادة في محتوي االمواد الأيضيه الثلاثه – موضوع البحث- كانت كبيرة جدا في الصنف المقاوم للملوحة اذا ماقورنت بالصنف الحساس للملوحه ولقد تم مناقشة نتائج البحث في ضوء ما أجراه الأخرون من بحوث في هذا المجال و كذلك في ضوء الميكانيكيات المختلفه لمقاومة نبات الخس للملوحه