YYÜ TAR BİL DERG
(YYU J AGR SCI)
2013, 23(3): 209-214

Geliş Tarihi (Received): 17.01.2013
KabulTarihi (Accepted): 03.05.2013

Araştırma Makalesi/*Research Article (Original Paper)*

Identification of Ion Accumulation and Distribution Mechanisms in Watermelon Seedlings ((*Citrullus lanatus* (Thunb.) Mansf.) Grown under Salt Stress

Fikret YASAR[1]*, Ozlem UZAL[1], Ozlem YASAR[2]

[1]Faculty of Agriculture, University of Yuzuncu Yil, Zeve Campus, Van, Turkey
[2]Ozalp Vocational High Schools, Yuzuncu Yil University, Ozalp/Van, Turkey
* e-mail: fyasar@yyu.edu.tr phone: + 90 532 511 92 65, fax: + 90 432 225 11 04

Abstract: The aim of this study was to determine ion accumulation and distribution mechanisms in watermelon. Salt sensitive Golden crown F1 and salt tolerant Midyat genotype Watermelon (*Citrullus lanatus* (Thunb.) Mansf.) seedlings were grown in Hoagland nutrient solution. When plants had 3-4 true leaves, 100 mM NaCl was applied. Shoot fresh weights, Na^+, K^+, Ca^{+2} ion distribution in root, stem and leaves of the plants subjected to 14 days salt stress were determined. Salt tolerant Midyat genotype had higher shoot fresh weight than salt sensitive Golden crown F_1 variety. Ion (Na^+, K^+, Ca^{+2}) accumulation in Golden crown F_1 variety root was higher than Midyat genotype. Nevertheless, while Na ion accumulation in the shoot of the Golden Crown F_1cultivars were higher, no differences between genotypes were determined for K^+, Ca^{+2} ions. Na was higher in salt sensitive variety's leaves, whereas, K^+ and Ca^{+2} ions accumulation was higher in Midyat genotype's leaves. As a result, it was determined in this study that salt avoidance mechanism worked in watermelon. Additionally, it was observed that watermelon acted to be selective in ion uptake, there was a competition in terms of K^+ and Na^+ ions uptake and this salt-tolerant genotype had higher K^+ accumulation rate. There was an increase in K accumulation in salt stress treated plants. A reverse relationship between K and Na ions was determined in ion distribution among organs. While N accumulation in an organ increased K ion accumulation decreased in that organ, and vice versa. Accumulation of Ca ions increased from root of the plant towards to leaves.

Key words: Ion accumulation, Ion distrubition, Organ, Salt stress, Watermelon

Tuz Stresi altında Yetiştirilen Karpuz Fidelerinde İyon Birikim ve Dağıtım Mekanizmalarının ((*Citrullus lanatus* (Thunb.) Mansf.) Belirlenmesi

Özet: Tuza hassas Golden crown F_1 ve tolerant yerel Midyat genotipine ait karpuz (*Citrullus lanatus* (Thunb.) Mansf.) fideleri Hoagland besin çözeltisi içerisinde büyütülmüştür. Bitkiler 3-4 gerçek yaprağa sahip iken 100 mM NaCl uygulanmıştır. 14 gün süreyle tuz stresi uygulanan bitkilerin yeşil aksam ağırlıklarına, kök, gövde ve yapraklarındaki Na, K, Ca iyon dağılımlarına bakılmıştır. Çalışmadaki amaç karpuzda iyon birikim ve dağılım mekanizmasını belirlemektir. Tuza toleranslı olan Midyat genotipinin yeşil aksam yaş ağırlığı, hassas olan Golden crown F_1 çeşidine göre daha yüksek çıkmıştır. Ayrıca Golden crown F_1 çeşidinin bitkilerinin köklerindeki iyon birikimleri (Na, K, Ca) Midyat genotipinden daha yüksek bulunmuştur. Fakat Golden crown F_1 çeşidinin gövde kısmında biriktirdiği Na iyonu yüksek çıkarken, K^+, Ca^{+2} iyonları bakımından genotipler arasında fark bulunmamıştır. Yapraklarda ise Na^+ iyonu yine hassas olan çeşitte yüksek, toleranslı Midyat genotipinde düşük bulunmuş, Midyat genotipinde K^+ ve Ca iyonları birikimi daha yüksek çıkmıştır. Sonuç olarak bu çalışmada gözlemlenen, karpuzda tuzdan sakınım mekanizmasının çalıştığıdır. Ayrıca, karpuzun iyon alımında seçici davrandığını, K ile Na arasında alınım bakımından bir rekabetin olduğunu ve tolerant genotiplerin yüksek oranda K tutma kapasitesine sahip olduklarını gösterdiği gibi, tuz stresi uygulanmış bitkilerin K birikimlerinde artışın olduğu görülmüştür. Organlara göre iyon dağılımında, Na iyonu ile K iyonu zıt ilişki göstermiştir. Na iyonunun arttığı organda K azalırken, Na'nın az biriktiği organda K iyonu artmıştır. Ca iyonun bitkinin köklerinden yapraklarına doğru birikimi artmıştır.

Anahtar kelimeler: İyon birikimi, İyon dağılımı, Organ, Tuz stresi, Karpuz

Introduction

Plants develop certain defence mechanisms against all kinds of stress factors caused by biotic and abiotic resources in the nature and they endeavor to continue to grow and develop by adapting themselves to negative circumstances. Levitt (1980), who provided the first important information about salt stress in plants and their reactions against it, mentions that the resistance against the stress caused by salt is controlled by the mechanisms of avoidance of salt and/or tolerance to salt. Tal (1983) and Lauchli (1986) have also adopted this view and say that plants keep salt permeability low in their stem cells for keeping salt away from their trunks in the mechanism of avoidance of salt. Stem cells of the plants resistant against salt are expected to have impermeability as much as possible. Salt is kept away through a passive attitude of the cell. Also, the plants can pump Na ions out of the cell for expelling salt under the extent of the mechanism of avoidance of salt. The fact that Na ions are driven out of the cytoplasm through Na pumps allows that Na amount in the plant stays within tolerable limits (Yang et al. 1990). Thus, we may mention about an expelling mechanism requiring energy use besides passively keeping salt away (Hasegawa et al. 1986). Another method is to reduce the accumulated salt in unit volume by rapid growing, in other words, to dilute salt within the body. Salt ions accumulated in cells may be driven out by arresting them in vacuoles in another mechanism and then, plants are protected against harmful effects of salt (Tattini et al. 1994; Zhang et al. 2001).

Faced with salt stress in plants, from the outside to be in strengthening the salt ions or endogenously synthesized soluble organic substances and their accumulation in the cell can be achieved through osmotic adjustment (Marschner 1995). The term osmotic adjustment in plants, salinity, or to a lack of water, ions, free amino acids and soluble sugars by the accumulation of active osmotic potential is defined as a balance (Gabor et al. 1986). Another term in the osmotic adjustment of plants, some inorganic ions such as K and Na or glycerol, sucrose, proline, betaine is defined as the ability to accumulate organic matter, such as (Hellebust 1976). Water and other environmental stresses in plants exposed to proline accumulation has been observed frequently (Hanson and Hitz 1982). Proline under osmotic stress increasing level of NaCl is thought to play a role in editing (Joyce et al. 1992). Plants tolerate salt by restricting Na ion migration from roots to stems and leaves (Poljakoff –Mayber 1975).

The purpose of this study was to explain the mechanism of salt stress by the ion disribution/accumulation in the root, stem and leaves of the watermelon genotypes which previously determined as tolerant (Midyat) and sensetive (Golden Crown F_1) (Yasar et al. 2006 and Yasar et al. 2007). In the previous study (Yasar et al. 2006 and Yasar et al. 2007), we determined that the salt-sensitive and tolerant Midyat (tolerant) genotypes with the Golden Crown F_1 (susceptible) varieties in order to fully explain the mechanism of salt stress, ion accumulation of the plant's roots stems and leaves were examined separately.

Material and Methods

Plant Material

Salt-sensitive cultivar ‘Golden Crown F_1’ and salt-tolerant local variety ‘Midyat’were employed as plant materials in this study (Yasar et al. 2006 and Yasar et al. 2007).

Growing plants and salt application

Watermelon seeds were sown in the pots made of foam with a hole in their bottoms and filled with pumice and they were germinated in the climatic room at the temperature 25^{o}C and under the relative humidity of 65%.

After the seeds had been germinated and their first true leaves had been formed, the seedlings were irrigated with Hoagland nutrient solution (Hoagland and Arnon 1938). The seedlings were transferred to plastic vessels filled with 4 L Hoagland’s solution after their second true leaves had been formed in the pumice germination medium. Nutrient solutions were refreshed once a week and vessels were re-positioned for ensuring that all plants benefit equally from lightening conditions. Seedlings grown in nutrient solution Hoagland medium, after the formation of 4-5 true leaves, 100 mm NaCl was applied gradually. First day of 50 mM NaCl, the second day, 50 mM NaCl was applied. The nutrient solution renewed every week, provided that the same concentration of salt applied.

Mineral element analyses

The plants were divided into the sections of root, stem and leaves after they had been weighted. The samples collected from the plants were put into glass jars and stored in a deepfreeze at –40°C for the analyses to be done in the future. 250 mg sample was taken from each plant parts sample. 15 ml of 0.1 N HNO_3 (Nitric acid) and a drop of toluene were added onto it and they within closed plastic boxes were left to wait for one week in dark and at the end of this period, the samples were shaken for 24 hours on a shaker. Fresh weight recorded after NaCl aplication at 0 mM and 100 mM concentrations had been applied to Midyat watermelon variety tolerant to salt stress and Golden Crown F_1 hybrid cultivar sensitive to salt stress and accumulated Na, K, Ca ion values in their roots, stems and leaves were examined to determination ion distribution mechanisms of these tolerant and sensitive genotypes. Na^+, K^+ and Ca ions in these extracts, which had been prepared in this way, were measured through flame photometric method with the help of Eppendof flame photometer. Ion amounts in the fresh plant samples were determined as fresh weight (μg/mg Fresh Weight) (Taleisnik et al. 1997). Relative ion amount in 100 g of plant parts was calculated by summing Na, K and Ca ions in all parts of the plant measured as μg/mg fresh weight.

Results and Discussion

Growing rates of both salt-tolerant variety and salt-sensitive cultivar were reduced in hydroponic culture under salt stress compared with the control group. However, this decline is higher in case of salt-sensitive cultivar. It was found that the plants of Midyat variety were very strong and this genotype showed high performance under control and salt stress conditions compared with F_1 cultivar (Table 1). Similar results were obtained in our previous study including these watermelon genotypes (Yasar 2006, Yasar et al. 2007) and other studies on watermelon. Furthermore, Yasar (2003) reported that salt-tolerant eggplant genotypes showed higher growth performance than that shown by the others.

Table 1: Fresh weights of watermelon plants in the control and salt applied (g $plant^{-1}$)

Genotype	Shoot Fresh Weight	
	0 mM NaCl	100 mM NaCl
Golden crown F1	2.007 b* A*	0.865 b B
Midyat	6.738 a A	3.432 a B

*Different means case represents different means of genotypes in the same dose level, (comparison of genotypes)
*Different letter case represents different means of genotypes in the same dose level, (comparison of genotypes)

Accumulated K or Na ion distribution based on the organs was the factor determining resistance against salt stress in case of watermelon. Especially, if the plant failed to be selective in driving the ion collected from the root to the growing end or failed to send the ions proportionally, the decline in its growing rate was higher. Similar situation were found by Wolf et al. (1991) in barley, by Perez-Alfocea et al. (1993) in tomato, by Taleisnik and Grunberg (1994) in tomato, and by Yasar et al. (2006) in fresh bean through their studies.

Concerning Na accumulations in the watermelon seedlings under salt-stress, more Na was accumulated in all organs of both tolerant and sensitive genotypes compared with the control plants. Na accumulation in root, stem and leaves of Midyat local variety is approximately half of the Na amount accumulated in root, stem and leaves of Golden crown F_1 cultivar. The amounts in the root and the stem of Golden crown F_1 were the same and the amount in the leaves was lower. The amounts of Na accumulated in the root and in the leaves of Midyat variety were similar; however, the amount in the stem was higher (Table 2).

Table 2: Na^+, K^+ and Ca^{2+} ions concentration (µg mg^{-1} DW(Dry Weight)) in roots, stems, and leaves of control (0 mM NaCl) and salt-treated (100 mM NaCl) watermelon seedlings

Ions	NaCl addition	Genotype	Root	Stem	Leaf
Na	**0 mM**	Golden crown F_1	18.28 a* B* I*	14.54 a B II	11.69 a B III
		Midyat	18.82a B I	14.35 a B II	9.68 b B III
	100 mM	Golden crown F_1	268.53 a A I	268.69 a A I	238.94 a A II
		Midyat	128.64 b A II	157.03 b A I	120.41 b A II
K	**0 mM**	Golden crown F_1	1.36 a A I	1.17 b B II	1.11 a B III
		Midyat	1.14 b B II	1.38 a B I	1.09 a B III
	100 mM	Golden crown F_1	1.38 b A I	1.40 b A I	1.50 b A I
		Midyat	2.10 a A I	1.74 a A II	1.96 a A I
Ca	**0 mM**	Golden crown F_1	0.071 a B III	0.13 b b A II	0.241 a A I
		Midyat	0.083 a A III	0.205 a A I	0.131 b B II
	100 mM	Golden crown F_1	0.110 a A III	0.156 a A II	0.202 b B I
		Midyat	0.056 b B III	0.165 a B II	0.358 a A I

*Different letter case represents different means of genotypes in the same dose level, (comparison of genotypes)
*Different upper case represents different means of organ in the same genotypes and dose level, (comparison of organs)
*Different numbers represents different means dose level in the same genotypes and organs, (comparison of doses)

Wolf et al. (1991) in th a study on barley, Soliman and Doss (1992) in a study on tomato, Perez et al. (1993) in a study on tomato, Cuartero and Fernandez-Munoz (1999) in a study on tomato, Santa-Maria and Epstein (2001) in a study on wheat and Yasar et al.(2006) in a study on bean reported that Na ion distribution in various organs and tissues of green parts was very important in the varieties tolerating salt. These researchers reported that Na ion causing salt stress was attached to mostly older leaves and its migration through younger leaves was restricted and this is one of the best features of the salt-tolerant plants known until today. It was observed in this study that salt avoidance mechanism worked in salt tolerant watermelon genotype. Also, although declines in K accumulations of many plants occurred after salt application in the studies done by many researchers on many species (Yasar 2003; Kusvuran et al. 2006), an increase was seen in case of watermelon. The highest increase occurred in salt-tolerant Midyat variety. This evidences that watermelon behaves selective in ion intake, K and Na compete for being taken and tolerant genotypes have high K arresting capacity. Similar results have been achieved by Caro et al (1991), Cuartero et al (1992), Perez-Alfocea et al (1993), Taleisnik, and Grunberg (1994), Zhang and Blumward (2001) and Dasgan et al (2002) in their studies done on various tomato genotypes. Ca accumulation in the roots of the plants was low in salt-tolerant variety under salty conditions. However, Ca accumulation in the leaves of the same variety was quite high. Ca accumulation in the stem was similar in both genotypes (Table 2). Similar results have been found by Cramer et al. (1986), Huang and Redman (1995), Lauchli (1990), Rengel (1992), Cuartero et al. (1992), Zhang and Blumward (2001) and Dasgan et al. (2002) in their studies done on various plants.

Conclusion

The development of Midyat genotype in both control and salt treatment groups was better than Golden crown F_1. Ion accumulations in both genotypes' roots, stems and leaves were similar in general, however, ion accumulation changed with salt treatment. When ion distribution among organs was evaluated it was noted that lesser Na ion accumulation occurred in leaves of salt tolerant Midyat genotype. Additionally, K ions, competing with Na ion, accumulated more. Accumulation of Ca ions increased from root of the plant towards to leaves. Since the highest ion accumulation occurred in leaves the plants did not get harmed.

Acknowledgements

This study was supported by Scientific Research Projects of Yuzuncu Yil University.

References

Caro M, Cruz V, Cuartero J, Estan MT, Bolarin MC (1991). Salinity tolerance of normal-fruited and cherry tomato cultivars. Plant and Soil, 136: 249-255.

Cramer GR, Lauchli A, Epstein E (1986). Effects of NaCl and $CaCl_2$ on ion activities in complex nutrient solutions and root growth of cotton. Plant Physiol., 81:792-797.

Cuartero J, Fernandez-Munoz R (1999). Tomato and salinity. Sci. Hort.,78:83-125.

Cuartero J, Yeo AR, Flowers TJ (1992). Selection of donors for salt-tolerance in tomato using physiological traits. New Phytol. 121: 63-69.

Dasgan HY, Aktaş H, Abak K, Cakmak I (2002). Determination of screening techniques to salinity in tomatoes and investigation of genotype responses. Plant Science, 163: 695-703.

Gabor G, Simon-Sarkadi L, Bekes F, Erdei L (1986). Genotype specific chances in amino acid and polyanime of wheat tissue culture induced by osmotic stress. Advences in Agricutural Biotecnology, 170-176.

Hanson AD, Hitz WD (1982). Metabolic responses of mesophytes to plant water deficits: Ann. Rev. Plant Physiol., 33, 163-203.

Hasegawa PM, Bressan RA, Handa AV (1986). Cellular mechanisms of salinity tolerance. Hort. Sci., 21: 1317-1324.

Hellebust JA (1976). Osmoregulation. Ann. Rev. Plant Physiol., 27: 485-505.

Hoagland DR , Arnon DI (1938). The water culture method for growing plants without soil. Circ. Calif. Agr. Exp. Sta., 347-461.

Huang J, Redman RE (1995). Solute adjustment to salinity and calcium supply in cultivated and Wild Barley. J. Plant Nutrition, 18: 1371-1389.

Joyce PA, Aspinall D, Palley LG (1992). Photosynthesis and the acumulation of proline in responce to water deficit. Aust.J.Plant.Physiol.,19:249-261.

Kuşvuran Ş, Yaşar F, Abak K, Ellialtıoğlu Ş (2006). Under salt stress grown melon (*Cucumis melo* L.) genotypes ion accumulation in leaves and the relationship between salt tolerance. IV. Symposium of vegetable cultivation, 19-22 September 2006, K.Maraş.395-398.

Lauchli A (1986). Responses and adaptation of crops to salinity. Acta Hort., 190: 243-246.

Lauchli A (1990). Calcium, salinity and plasma membrane. In: calcium in plant growth and development (R.J. Leonard and P.K.Hepler eds), The American Society of Plant Physiologists Rockville, MD., 26-35.

Levitt J (1980). Responses of plants to environmental stresses. Vol.II, 2nd ed. Academic press, New York, pp:607.

Marschner H (1995). Mineral Nutrition of Higher Plants. Academic Press, 657-680.

Perez-Alfocea F, Estan MT, Caro M, Bolarin MC (1993). Response of tomato cultivars to salinity. Plant Soil. 150:203–211.

Poljakoff-Mayber A (1975). Morphological and anatomical changes in plants as response to salinity stress. In: Plant in salinine environments. Pollakoff-Mayber, A. and Gale, J. (eds.) Springer-Verlag, New York, U.S.A. pp.98-117.

Rengel Z (1992). The role of calcium in salt toxicity. Plant and Soil., 229-233.

Santa-Maria GE, Epstein E (2001). Potassium/ Sodium selectivity in wheat and the amphiploid cross wheat x Lophopyrum elongatum. Plant Sci., 160: 532-534.

Soliman MS, Doss M (1992). Salinity and mineral nutrıtıon effects of growth and accumulation of organic ions in two cultivated tomato varietes. J. Plant Nutrition, 15 (12): 2789-2799.

Tal M (1983). Selection for stress tolerance. In ''Handbook of Plant Cell Culture, Volume 1'' (D.E. Evans, W.R. Sharp, P.V. Ammirato, Y. Yamada, eds.), Collier Macmillan Publishers, London, 461-487.

Taleisnik E, Grunberg K (1994). Ion balance in tomato cultivars differing in salt tolerance. I. Sodium and potassium accumulation and fluxes under moderate salinity. Physiologia Plantarum, 92:528-534.

Taleisnik E, Peyrano G, Arias C (1997). Respose of Chloris gayana cultivars to salinity. 1. Germination and early vegetative growth. Trop. Grassl. 31: 232-240.

Tattini M, Coradeschi MA, Ponzio C, Traversi L (1994). Responses of olive plants to salt stress. Abstracts. XXIVth Int. Hort. Congress**,** 21-27 August 1994, Kyoto-Japan ISHS.

Wolf O, Munns R, Tonnet M, Jeschke WD (1991). The role of the stem in the partitioning of Na^+ and K^+ in salt-treated barley. J. of Exp. Bot., 42:278-282.

Yang YW, Newton RJ, Miller FR (1990). Salinity tolerance in sorghum. I. Whole plant response to sodium chloride in S.bicolor and S. halepense. Crop Sci., 30: 775-781.

Yasar F (2006). Effects of salt stress on ion and lipid peroxidasion content in greenbeans genotypes. Asian Journal of Chemistry, 19, (2), 1165-1169.

Yasar F (2003). Some of antioxidant enzyme activity investigation as in vivo and in vitro of eggplant genotypes under salt stres. Yüzüncü Yıl University Inst. of Science, Van, 138 s.

Yasar F, Ellialtıoğlu Ş, Özpay T, Üzal Ö (2007). Under salt stress of watermelon (*Citrullus lanatus* (Thunb.) Mansf.) Identification of genotypic differences. Vth Congress of Horticultural Science, 4-7 September 2007, Erzurum, 67-71.

Yaşar F, Özpay T, Üzal Ö , Ellialtıoğlu Ş (2006). Determination the response of watermelon to salt stress IVth Symposium of Vegetable, 19-22 September 2006, K.Maraş, 250-252.

Zhang HX, Blumward E (2001). Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. Nature Biotecnology**.** 9:765-768.