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In vitro Şartlar Altında 'Bursa Siyahı' (*Ficus carica* L.) İncir Çeşidinin Morfolojisi Üzerine Tuzun Etkisi

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ÖZET

Bu çalışmanın amacı *Ficus carica* cv. Bursa Siyahı fidelerinin morfolojisi üzerine farklı NaCl konsantrasyonlarının etkisini belirlemektir. *Ficus carica* L. cv Bursa Siyahı tohumları *in vitro* şartlar altında çimlendirilmiş ve *in vitro* olarak elde edilmiş fideler tuz stresi için farklı konsantrasyonda NaCl içeren ve kontrol olarak NaCl içermeyen Murashige ve Skoog ortamında kültüre alınmıştır. Bitki canlılığının, ortamdaki NaCl konsantrasyonunun artışına bağlı olarak azaldığı gözlemlemiştir. Altı haftanın sonunda, bitkiler taze ağılık (TA), sürgün uzunluğu (SU), yaprak sayısı (YS), kök sayısı (KS) ve kök uzunluğu (KU) gibi parametrelerle değerlendirilmiştir. 40 mM NaCl ilaveli ortamda bulunan bitkiler maksimum büyüme göstermişlerdir, tuz stresi etkilerinin artan tuz konsantrasyonlarına bağlı olarak arttığı gözlemlenmiştir.

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Araştırma Makalesi

Effects of Salinity on the Morphology of the ' Bursa Siyahı', Fig (*Ficus carica* L.) cultivar *In vitro* Conditions

ABSTRACT

The objective of this study was to examine the effects of NaCl on the morphology of *Ficus carica* cv. 'Bursa Siyahı' seedlings. For the salt stress tolerance, seeds of *Ficus carica* L. cv 'Bursa Siyahı' were germinated *in vitro* conditions and obtained seedlings were cultured in Murashige and Skoog culture medium without NaCl (as control) and with different NaCl concentrations. It was observed that plant viability were decreased with increasing NaCl concentration in the medium At the end of six weeks, plants were evaluated for the parameters of fresh weight (FW), shoot length (SL), leaf number (LN), root number (RN) and root length (RL). Plants in medium with 40 mM NaCl gave the maximum growth, salt stress effect was found to be increased in relative to the salt concentrations.

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INTRODUCTION

Each plant needs special environmental conditions to continue its viability. Development and growth depend on environmental biotic and abiotic factors. One of the main problems exiting in soil in relation to plant growth (Stepien and Johnson, 2009) is abiotic factors. Abiotic stresses such as salt excess and drought are among factors most limiting to plant productivity (Bohnert et al., 1995).

Salinity affects growth and development of plants through oxidative, ionic and osmotic stresses, and spoils normal growth and development of plant (Yeo and Flowers, 1983; Noble and Rogers, 1992). Excessive salt concentration in the soil limits plant growth in three ways; first it leads to decreased osmotic potentials. So it reduces the water availability of the external solution, which can affect the water status of the plant (Golombek and Lüdders, 1993). Second, ions such as sodium and chloride in the plant can lead to toxicity or interfere with the uptake process. Thirdly, the concentration of sodium and chloride or excessive salt in the soil can impair the absorption and transportation of other essential nutrients from the soil. This situation is called as nutritional imbalance (Kamrani et al., 2013).

Soil salinity is a major abiotic stress factor (Zhu, 2001) and limiting factor for the plants in arid and semi-arid zones of the world with low rainfall or irrigated with salty water. Most of the screening experiments for salt tolerant genotypes were conducted under either *in vitro* or controlled environmental conditions (Kingsbury and Epstein, 1984; Rawson et al., 1988; Barakat and Abdel Latif, 1996; Arzani and Mirodjagh, 1999; Munns et al., 2000).

Ficus carica L. (fig) belongs to *Moraceae* family, which has an economical importance in Turkey.

Fig fruits called anjeer has nutritive and medical values. It is known to be used in various drug formulations for its mild laxative action and high alkalinity (Kirtikar and Basu, 1986).

The cultivation of the fig is practiced under irrigation. Although fig is a moderately salt-tolerant species, its tolerance limit is unknown in increasing saline conditions. Because the fig is generally grown on saline soils, salinity problems are becoming more important.

There is only one study on the short-term salinity effects on two varieties 'Bardajik' and 'Faro' of *Ficus carica* L. (Golombek and Lüdders,1993). But, there is no any studies on the effect of different salt concentration on the morphology of *Ficus carica* L. at *in vitro*. Therefore, this study aims to determine the morphological response of the *Ficus carica* cv. 'Bursa siyahı' to salt *in vitro* conditions.

MATERIALS and METHODS

In this study, seeds of *Ficus carica* L. cv. 'Bursa Siyahı' were used as initial plant source. Seeds were isolated from mature fruit in the summer. For pre-treatment applications, they were cleaned in running top water for an hour and were surface-sterilized with 70 % EtOH for 10 min and with 4.5 % sodium hypochlorite for 15 min, consecutively, and washed three times with sterilized distilled water. Then seeds were maintained on liquid basal medium with paper bridge jars. Basal medium contained Murashige & Skoog (Murashige and Skoog, 1962) mineral salts, 100 mg l^{-1} myo-inositol, 2 mg l^{-1} glycine, 0.5 mg l^{-1} nicotinic acid, 0.5 mg l^{-1} pyridoxine HCl, 0.1 mg l^{-1} thiamine HCl, 30 g l^{-1} sucrose. The pH of the media was adjusted to 5.8 before autoclaving at 121°C for 15 min. Agar-agar (8 g L^{-1}) were added to the media as gelling agent. The experiments were conducted with three replicates consisting of ten explant per jars and repeated three times.

In order to determine the plant response to salt medium, seedlings obtained from germination of seeds were transferred onto medium without or with NaCl. MS medium were used as control to compare the effects of different concentrations of NaCl salt (40, 80, 120, 240, 320, 480 mM). The experiments were conducted with ten replicates consisting of two seedling per jars and repeated three times.

Plants survived after salt stress were evaluated by recording parameters of fresh weight (FW), shoot length (SL), leaf number (LN), root number (RN) and root length (RL).

All cultures were maintained in a culture room at 24±1ºC with a photoperiod of 16 h light under a light intensity of 30 $\mu Em^{-2}s^{-1}$.

Statistical analysis

Data of survival ratio (%) of the plants were evaluated. The data fresh weight (FW), shoot length (SL), leaf number (LN), root number (RN) and root length (RL) were analyzed as mean ± SE (standard error) value. The statistical differences were estimated at the 5% level by the Duncan's Multiple Range Test.

RESULTS and DISCUSSION

In tissue culture studies, seeds are preferred starting materials (Fay, 1994). In addition, *in vitro* germinated seeds supply aseptic seedlings can then be used in tissue culture applications. According to the results of this experiments, the germination rate of seeds was found to be 100 %. Following emergence of the radicula, the plants were transplanted to MS medium without or with different concentrations of NaCl. In the second week, according to the morphological observations, the plants in control medium and medium with 40 and 80 mM NaCl did not showed any stress symptoms (Table 1). However, brown leaf edge and chlorosis were observed due to increasing salt concentrations. Survival rate of the plants were decreased in medium with 120 mM NaCl and the plants in medium with 480 mM NaCl were lost (62.5%). Also, the leaf number were gradually decreased due to increasing salt concentration (Table 1).

Almost all the plantlets in medium of higher concentrations of NaCl (320 and 480 mM) were died after a period of time. Therefore, plantlets in lower concentrations of NaCl (0-240 mM) were evaluated for the parameters of fresh weight (FW), shoot length (SL), leaf number (LN), root number (RN) and root length (RL) end of the six weeks of culture (Table 2).

According to the results, the lower concentrations of NaCl and control showed positive effects on the number of leaves, fresh weight, root number and root length while the increasing salt concentrations gave negative effects.

Also, the highest value of fresh weight (FW) was obtained from the plantlets in medium of 40 mM NaCl (Table 2, Fig 1), while this value was decreased in higher concentrations of NaCl.

Table 1. Morphological appearance and survival rate after two weeks of culture.

NaCl (mM)	Morphological appearance	Survival rate (%)	Numbers of leaf mean ±SE (standard error)
0 (control)	Healthy	100	6.06 ± 0.45
40	Healthy	100	6.00 ± 0.33
80	Healthy	100	5.50 ± 0.20
120	Beginning of browning	93.75	5.38 ± 0.30
240	Beginning of chlorosis	87.5	5.31 ± 0.31
320	Chlorosis	50.0	2.63 ± 0.72
480	Chlorosis	37.5	2.19 ± 0.76

Values are means of repeated experiments ± standard error.

Farhoudi et al. (2015) reported that root length and seedling fresh weight decreased with increasing salinity. The most typical symptom of saline injury to a plant is retarded growth due to inhibition of cell elongation (Bandeoglu et al., 2004). Formed osmotic and drought stress with salt accumulation in plant lead to decrease of water absorption by plant tissues. Therefore cellular growth and development decrease. Thus, inhibition of water absorption and its effect on cellular growth and development is one of the most important causes of decreased growth of stem and root (Cavalcanti et al., 2007; Munns, 2002).

Considering the terms of the number of leaves of the plants grown in 40 mM NaCl medium and control gave the best values (Table 2.) Golombek and Lüdders (1993) have reported that salt was reduced the leaf area of *Ficus carica* cv 'Bardajik' and *F. carica* cv 'Faro'. There was a decrease in the number of roots depending on the increasing salt concentrations. It was seen that the number of roots were affected negatively even at low salt concentrations. Considering the root length the control medium was found to be the best medium. Golombek and Lüdders (1993) have reported that the root mass after one week of NaCl (100 mM) treatment of *Ficus carica* cv 'Bardajik' was higher than those of control. They were also stated that root mass after one week of NaCl (100 mM) treatment of *Ficus carica* cv 'Faro' was lower than those of control.

Table 2. Morphological observation of plantlet on medium without and with NaCl after 6 weeks of culture.

NaCl (mM)	FW(g)	LN mean± SE	RN ±SE mean± SE	SL ± SE (cm) mean± SE	RL ± SE(cm) mean± SE
0 (control)	0.20 ab	8.94 ± 0.76 a	8.25 ± 1.08 a	4.80 ± 0.21 a	7.35 ± 1.14 a
40	0.24 a	9.06 ± 0.67 a	4.81 ± 0.92 b	4.16 ± 0.21 ab	4.61 ± 0.48 ab
80	0.16 bc	6.31 ± 0.22 b	2.81 ± 0.31 c	3.85 ± 0.18 b	4.20 ± 0.46 b
120	0.14 c	6.31 ± 0.50 b	2.56 ± 0.22 c	3.83 ± 0.24 b	5.79 ± 0.80 b
240	0.13 c	4.94 ± 0.38 b	2.44 ± 0.20 c	3.77 ± 0.23 b	4.47 ± 0.63 b

Means followed by same letters are not significantly different by the Duncan Multiple Range Test ($p < 0.05$). Values are means of repeated experiments ± standard error.
FW: fresh weight, **SL**: shoot length, **LN**: leaf number, **RN**: root number, **RL**: root length.

The length of the shoots were negatively affected due to the increasing salt concentration. As shown in the table 2, the seedling development were affected negatively due to increasing salt concentration more than 40 mM NaCl. The effect of salt on plants were firstly appeared at the lower leaves of the plantlets as yellowing and brownish, and followed by chlorosis and death (Fig 2 and Fig 3). Yadav et al. (2001) reported that plant growth parameters such as plant height decreased with increasing salinity. However, Kamrani et al. (2013) and Farhoudi et al. (2015) reported that plant height decreased with increasing salt concentration. These results were paralleled with my findings. This is probably results of the insufficient water uptake in the plant with the increase of osmotic pressure, blocking the uptake of other nutrients of salts, or breakdown the metabolic balance because of the excessive salt (Salisburry and Ross, 1992; Pandey and Kalloo, 1993). Hussain et al., (2004) were reported that reduced plant height in salty conditions is due to genetic nature.

CONCLUSION

Although figs are salt tolerant plants, plants which were exposed to higher salt concentrations because of the changes in rainfall or irrigation in the field conditions will cause damage in formation or even loss of vitality. Salt tolerance limitations of the figs has been not known until today. This is the first report on the effects of NaCl concentrations on the morphology of *Ficus carica* cv. 'Bursa Siyahı' seedlings. It is believed that, this report will help further upcoming studies on salinity effect on *Ficus carica* cv. 'Bursa Siyahı'.

Figure 1. Plantlet on MS medium with 40 mM NaCl after 6 weeks of culture.

Figure 2. Plantlet on MS medium with 120 mM NaCl after 6 weeks of culture.

Figure 3. Plantlet on MS medium with 240 mM NaCl after 6 weeks of culture.

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REFERENCES

Arzani A, Mirodjagh SS 1999. Response of Durum Wheat Cultivars to Immature Embryo Culture, Callus Induction and *In vitro* Salt Stress. Plant Cell Tiss. Org. Cult., 58: 67-72.

Bandeoglu E, Eyidogan F, Yucel M, Avni Oktem H 2004. Antioxidant Responses of Shoots and Roots of Lentil to NaCl-Salinity Stress. Plant Growth Regulation, 42: 69-77.

Barakat M, Abdel Latif H 1996. *In vitro* Selection of Wheat Callus Tolerant to High Levels of Salt Plant Regeneration. Euphytica, 91: 127-140.

Bohnert HJ, Nelson DE, Jensen RG 1995. Adaptations to Environmental Stresses. The Plant Cell,7:1099-1111.

Cavalcanti F, Lima JP,Silva S,Viegas R, Silveria J 2007. Roots and Leaves Display Contrasting Oxidative Response During Salt Stress and Recovery in Cowpea. J. Plant Physiol., 164:591-600.

Farhoudi R, Modhej A, Afrous A 2015. Effect of Salt Stress on Physiological and Morphological Parameters of Rapeseed Cultivars. J.Sci. Res. and Dev., 2(5):111-117

Fay MF 1994. In What Situation is *In vitro* Culture Appropriate to Plant Conservation? Biodivers Conserv, 3: 176-183.

Golombek SD, Lüdders P 1993. Effect of Short-term Salinity on Leaf Gas Exchange of the Fig (*Ficus carica* L.). Plant and Soil, 148: 21-27.

Hussain A, Khan ZI, Ashraf M, Rashid MH, Akhtar MS 2004. Effect of Salt Stress on Some Growth Attributes of Sugarcane Cultivars CP-77-400 and Coj- 84. Int. J. Agric. Biol., 6(1):188-191.

Kamrani MH, Hosseinniya H, Chegeni AR 2013. Effect of Salinity on the Growth Characteristics of Canola (*Brassica napus* L.). Tech. J. Eng and Applied Sci., 3(18):2327-2333.

Kingsbury RW, Epstein E 1984. Selection for Salt Resistant in Spring Wheat. Crop Sci., 24: 310-315.

Kirtikar KR, Basu BD 1986. Indian Medicinal Plants. International Book Distributors,Vol:3, Dehradun, 2329p.

Munns R, Hare RA, James RA, Rebetzke GI 2000. Genetic Variation for İmproving the Salt Tolerance of Durum Wheat. Aust.J. Agric. Res., 51: 69-74.

Munns R 2002. Comparative Physiology of Salt and Water Stress. Plant Cell and Environment, 25:239-250.

Murashige T, Skoog F 1962. A Revised Medium for Rapid Growth and Bioassays with Tabacco Tissue Cultures. Physiol. Plant., 15: 473-497.

Noble CL, Rogers ME 1992. Arguments for The Use of Physiological Criteria for Improving the Salt Tolerance in Crops. Plant Soil,146: 99-107.

Pandey SC, Kalloo G. 1993. Spinach, *Spinacia oleracea* L. In "Genetic Improvement of Vegetable Crops", (Ed. Kalloo G, Bergh BO), Pergamon Press, pp. 325–336, DOI: 10.1016/B978-0-08-040826-2.50027-8

Rawson HM, Richards RA, Munns R 1988. An Examination of Selection Criteria for Salt Tolerance in Wheat, Barley and Triticale Genotypes. Aust. J. Agric. Res., 39: 759-772.

Salisburry FB, Ross CW 1992. Plant Physiology. Wadsworth Pub. Com. Belmont, California, USA

Stepien P, Johnson NG 2009. Contrasting Responses of Photosynthesis to Salt Stress in The *Glycophyte arabidopsis* and The Halophyte *Thellungiella*: Role of The Plastid Terminal Oxidase as an Alternative Electron Sink. Plant Physiol., 149: 1154-1165.

Yadav AC, Mangel JC, Lal S, Sharma SK, Kapoor A 2001. Effect of Salinity and Phosphorus on Growth and Yield of Potato CV. Kufri Sutlej. Journal of Indian Potato Association, 28: 30-31.

Yeo AR, Flowers TJ 1983.Varietal Differences in The Toxicity of Sodium Ions in Rice Leaves. Physiol. Plant., 59:189-195.

Zhu J 2001. Plant Salt Tolerance. Trends Plant Sci., 6: 66-71.