EFFECTS OF SALINE STRESS ON IN VITRO SEED GERMINATION AND SEEDLING GROWTH OF SOME TURKISH FIG CULTIVARS (FICUS CARICA L.)

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Abstract. In this study, saline stress tolerance/sensitivity levels, germination rate of the seeds, and seedling growth of eight fig cultivars (Morgüz, Sarılop, Yeşilgüz, Sultan Selim, Halebi, Bursa Siyahı, Göklop and Izmir Bardacık) of Turkey were examined by in vitro screening. In fig, this is the first and guiding study reporting in vitro germination performance of the seeds in saline conditions for fig researchers. Salt stress in vitro condition was established by adding 0, 85.5, 171.1 and 342.5 mM NaCl to the MS basal medium in current experiment. In the examinations carried out 45 days after seedling, none of the cultivars could be obtained at a dose of 342.5 NaCl. Highest seed germination rates were determined in Sultan Selim (79.5%) and Bursa Black (75.0%) cultivars at 171.1 mM NaCl concentration. In other cultivars, this ratio has changed between 0.0-40.0%. High germination rate (90% <) and plant growth under the observed 85.5 mM NaCl dose was determined for Sultan Selim and Bursa Siyahı cultivars. The seeds and seedlings of Sarılop cultivar, one of the major cultivars for dried fig production, have been found sensitive to salt stress.

Keywords: fruit, fig (Ficus carica L.), salinity tolerance, stress physiology, germination criteria

Introduction

The fig, a subtropical fruit (Ficus carica L.) is grown in many parts of the world with mild climate. Turkey is one of the countries in the world with the largest share of producing and exporting figs. According to the latest reports, 305.7 thousand tons of fig are produced over 50.3 thousand hectares in Turkey. This value (1152.8 thousand tons) reaches 26.5% of the global fig production (FAO, 2017). Turkey is a very rich country in terms of the genetic resources of figs (Ercişli, 2004). Fig genetic diversity in Turkey is very valuable for classical and modern fig breeding programs. As indicated in previous studies, the genetic diversity found in cultivated plants and wild relatives provides rich resources for targeted cultivation characteristics and gene discovery that have not been used sufficiently yet (Ismail and Horie, 2017). It is important to reveal the genetic potential of the cultivars in terms of targeted character development. To evaluate genotypes in a short time, in vitro screening method can grant great convenience and time-saving. This technique has many advantages over ex vitro conditions to investigate the complex response of plants to stress factors as under aseptic and controlled environmental conditions (Jain, 2001; Lokhande et al., 2011; Rai et al., 2011). There are many studies in the literature that use these techniques for drought, salinity and other stress factors (Tal, 1994; Shibli et al., 2007; Shatnawi et al., 2009).
Figs are moderately tolerant (EC 3-5 dS m⁻¹) plants to the soil salinity (Mass and Hoffman, 1977; Golombok and Lüdders, 1994). The land where figs grow in the world usually contains above average saline levels. Therefore, salinity is a major problem for figs today and in the future (Golombok and Lüdders, 1994; Zarei et al., 2016; Soliman and Alhady, 2017). The result of dry and hot climatic conditions of the country, low precipitation and especially the improper irrigation practices implemented in the agricultural and landscaping applications, salinity problems arise in such areas where there are drainage problems. Salinity, one of the abiotic stress factors, negatively affects both the agricultural soil and the plants grown in the soil that is under the threat of salinity (Yılmaz et al., 2011). Against these stress factors, high yield producing and stress resistant species should be designated and cultivated in areas with salinity problem (Shannon, 1978; Epstein, 1985; Ashraf et al., 1986; Singh et al., 2018). Due to the expensive and difficult methods to eliminate the salinity problem in the cultivated land, salt-resistant plant species and the more tolerant genotypes of these species have been studied in recent years (Singh et al., 2018). Concordantly, seed reproduction has crucial value in the fig hybridization as well as in the seed reproduction mediated seedling production (Hartmann et al., 2011; Zarei et al., 2016; Nimbelkar et al., 2016).

In this study, seed germination and seedling growth performances of eight Turkish fig cultivars under salt pressure were resolved to effectively utilize in vitro screening technique. Likewise, the capability of germination of fig seeds under saline conditions has been demonstrated for the first time with this study by the wide application doses of NaCl and the susceptible/tolerance fig cultivars.

Materials and methods

Herein, mature seeds formed as a result of free pollination were used as vegetable material as Morgüz, Sarılop, Yeşilgüz, Sultan Selim, Halebi, Bursa Siyahı, Göklop, and İzmir Bardacık fig cultivars. For this purpose, the ripen fruits were obtained in season from Aydın Erbeyli Fig Research Institute located at 37° 51’ 56.0” – 27° 39’ 53.3” coordinates. First of all, the seeds are washed in the laboratory environment after being washed out and cleaned in a solution with sodium hypochlorite (5% active chlorine) with 0.1% (v/v). 20 g/L sucrose and 6 g/L agar containing hormone-free MS (Murashige and Skoog, 1962) medium were used as the main nutrient medium and the pH of the medium was set to 5.8. 0, 85.5, 171.1 and 342.5 mM NaCl were added to the basic nutrient medium. In the experiments, 25 ml medium in 100×15 mm petri dishes were used. 25 seeds were planted in each petri dish. While working with four replicas, the seeds were allowed to germinate for 45 days under saline stress. The cultures were incubated at 25 ± 1 °C under 16-h photoperiod with 35 μmol·m⁻²·s⁻¹ supplied by cool-white fluorescent lamps. The number of seeds germinated during the trial period was recorded daily. At the end of the experiment, germination rate (%), seedling height (mm), number of leaves (pieces/seedling rootstock) and root length (mm) were recorded.

Data were analysed according to Minitab Package Program (MINITAB Inc.). Analysis of variance (ANOVA) was applied with the F test (P < 0.05). Significant differences were determined based on a 5% error limit with Duncan test and differences were determined with the help of letters. Angle values of percentages were used in the analysis.
Results and discussion

In this study, the interaction between the germination rates, seedling height, number of leaves and root length in terms of various doses of NaCl was found to be statistically significant (Table 1). In terms of germination rate, the seeds of Sultan Selim, Bursa Siyahı, Göklo, and İzmir Bardacık were found to have statistically similar high germination rates at 0 mM and 85.5 mM NaCl concentrations. However, germination rates in Morgüz, Sarılop, Yeşilgüz and Halebi seeds decreased significantly compared to control in 85.5 mM application. Significant decrease in germination rates was recorded in 171.1 mM NaCl concentration. In this saline concentration, no germination occurred in Sarılop and Yeşilgüz seeds. However, germination rates of 79.5% and 75.0% were determined in the seeds of Sultan Selim and Bursa Siyahı respectively which are the highest values of 171.1 mM NaCl administration. No germination was noted at a concentration of 342.5 mM saline in any of the fig cultivars (Table 1).

Seedling height values decreased in all genotypes with increasing salt concentration. Moreover, the seedling height of Sultan Selim and Bursa Siyahı has the highest values compared to other cultivars in increasing saline concentrations (Table 1). The number of leaves in seedlings decreased significantly with elevated NaCl concentration in all cultivars. At 85.5 mM NaCl concentration, Sultan Selim, Bursa Siyahı and Halebi had a higher number of leaves than the others. There was no significant difference in the number of leaves between cultivars in 171.1 mM NaCl concentration (Table 1). The longest roots in 85.5 NaCl concentrations were recorded in Bursa Siyahı (52.4 mm), İzmir Bardacık (50.9 mm) and Sultan Selim (46.7 mm). The root length was significantly reduced in all cultivars at the 171.1 mM NaCl concentration. There was no significant difference among cultivars at this concentration (Table 1).

This is the first scientific study to reveal germination performances of fig seeds under saline stress. However, there are studies conducted on this subject in other woody plant species, albeit limited. Although all of these studies vary according to genotypes, germination rates and plant growth in seeds have been reported to be significantly decreased with an increasing salt concentration in the environment (Vijayan et al., 2003; Rahneshana et al., 2018). Similar findings were obtained in our study as well. Experiments showed that germination rates of fig seeds under saline stress manifested a great change depending on the type of cultivars and NaCl concentrations. In vitro conditions, the germination rate in fig cultivars on nutrient media (control- no NaCl) was 83-98%. At 85.5 mM (0.5%) NaCl concentration, the germination rate in fig cultivars was 10.3-99%, and at the highest NaCl concentration 171.1 mM (1%) the germination rate in fig cultivars was 0-79.5% (Table 1). NaCl causes an increase in osmotic pressure in the environment and prevents the seed from receiving water (Ardakani, 2015). NaCl leads to ionic stress as well as osmotic effect. It can also affect the activity of many enzymes that play a key role in lipid degradation (Zaghdoudi et al., 2015). In our study, one of the striking findings was that there was no germination in Yeşilgüz and Sarılop in the nutrient medium containing 171.1 mM NaCl. On the contrary, the same saline stress onto Sultan Selim and Bursa Siyahı has the highest germination rates 79.5% and 75.0% respectively. These results are vital for salinity resistant fig breeding since the elimination of the salinity originate problems depends on improvements of the cultivated genotypes salinity resistance (Athar and Ashraf, 2009).
Table 1. Effects of NaCl concentrations on in vitro seed germination and seedling growth of Turkish fig cultivars

| Cultivar          | 0 mM NaCl | 85.5 mM NaCl | 171.1 mM NaCl | 342.5 mM NaCl |
|-------------------|-----------|--------------|---------------|--------------|
|                   | (1) Germination ratio (%) |              |               |              |
| Morgüz            | 97.3 AB  | 64.0 D b     | 10.7 C c      | - A d        |
| Sarlıop           | 83.0 D A | 44.0 E b     | - D c         | - A c        |
| Yeşilgüz         | 84.0 D A | 10.3 F b     | - D c         | - A c        |
| Sultan Selim      | 98.0 A A | 99.0 A a     | 79.5 A b      | - A c        |
| Halebi            | 93.0 BCD A | 80.0 C b   | 40.0 B c      | - A d        |
| Bursa Siyahı      | 90.0 CD A | 91.2 B a     | 75.0 A b      | - A c        |
| Göklop            | 96.0 ABC A | 92.0 B a    | 36.0 B b      | - A c        |
| İzmir Bardakı      | 96.0 ABC A | 89.3 B C a | 9.3 C b      | - A c        |

(2) Seedling height (mm)

| Cultivar          |               |              |              |              |
| Morgüz            | 21.3 ± 0.7 D A | 7.2 ± 0.4 BC b | 4.0 ± 1.2 A c | -            |
| Sarlıop           | 24.8 ± 1.1 BC A | 7.4 ± 0.3 BC b | -            | -            |
| Yeşilgüz          | 18.4 ± 1.1 E A | 5.9 ± 0.7 C b | -            | -            |
| Sultan Selim      | 28.2 ± 2.0 A A | 15.5 ± 1.0 A b | 5.4 ± 0.3 A c | -            |
| Halebi            | 26.0 ± 1.0 AB A | 9.4 ± 0.6 B b | 5.3 ± 0.4 A c | -            |
| Bursa Siyahı      | 25.7 ± 2.3 ABC A | 13.7 ± 0.3 A b | 6.2 ± 0.8 A c | -            |
| Göklop            | 22.7 ± 1.4 CD A | 9.5 ± 0.2 B b | 4.8 ± 0.3 A c | -            |
| İzmir Bardakı      | 23.4 ± 2.0 BCD A | 7.6 ± 1.0 BC b | 4.4 ± 0.6 A c | -            |

(3) Leaf number

| Cultivar          |               |              |              |              |
| Morgüz            | 6.5 ± 0.4 CD A | 3.7 ± 0.1 CDE b | 1.8 ± 0.3 A c | -            |
| Sarlıop           | 5.7 ± 0.5 D A | 3.5 ± 0.2 DE b | -            | -            |
| Yeşilgüz          | 6.1 ± 0.5 D A | 3.1 ± 0.2 E b | -            | -            |
| Sultan Selim      | 8.6 ± 0.3 A A | 5.0 ± 0.2 A b | 1.7 ± 0.2 A c | -            |
| Halebi            | 7.1 ± 0.4 BC A | 4.4 ± 0.5 ABC b | 2.1 ± 0.1 A c | -            |
| Bursa Siyahı      | 7.8 ± 0.4 B A | 4.9 ± 0.1 AB b | 2.1 ± 0.1 A c | -            |
| Göklop            | 6.2 ± 0.1 D A | 4.1 ± 0.2 BCD b | 2.0 ± 0.1 A c | -            |
| İzmir Bardakı      | 6.4 ± 0.4 CD A | 3.8 ± 0.1 CDE b | 2.0 ± 0.1 A c | -            |

(4) Root length (mm)

| Cultivar          |               |              |              |              |
| Morgüz            | 48.1 ± 5.3 AB A | 34.4 ± 7.7 B b | 3.8 ± 0.6 A c | -            |
| Sarlıop           | 43.0 ± 5.0 BC A | 18.5 ± 5.6 C b | -            | -            |
| Yeşilgüz          | 58.4 ± 4.8 A A | 23.3 ± 5.7 C b | -            | -            |
| Sultan Selim      | 46.0 ± 1.3 BC A | 46.7 ± 0.7 A a | 4.7 ± 0.4 A b | -            |
| Halebi            | 35.0 ± 2.2 C A | 21.5 ± 3.2 C b | 3.4 ± 0.5 A c | -            |
| Bursa Siyahı      | 44.0 ± 1.7 BC A | 52.4 ± 5.1 A a | 3.7 ± 0.3 A b | -            |
| Göklop            | 39.1 ± 4.1 BC A | 34.6 ± 5.7 B a | 2.6 ± 0.4 A b | -            |
| İzmir Bardakı      | 39.7 ± 0.6 BC B | 50.9 ± 3.1 A a | 5.6 ± 0.8 A c | -            |

Source P-values

|            | (1) | (2) | (3) | (4) |
|------------|-----|-----|-----|-----|
| Cultivar (C) | 0.000 | 0.000 | 0.000 | 0.000 |
| Treatment (T) | 0.000 | 0.000 | 0.000 | 0.000 |
| C × T      | 0.000* | 0.016*** | 0.004** | 0.000* |

*For each parameter, different capital letters within a column indicate significant differences among cultivars in each treatment, and different small letters within a row show significant differences among treatments in each cultivar

*Data presented as mean ± standard error of mean

*Significant at P < 0.001, P < 0.01 and P < 0.05, respectively
The lack of germination of the seeds in the high saline conditions is the biggest factor limiting the obtaining of plants (Zaghdoudi et al., 2015). Procurement of water, retaining chloroplast function and ion homeostasis dictates the plant competence in enduring saline stress. In addition to those, osmotically controlling metabolites, some proteins, enzymes that confine free radicals play an important role in the plant homeostasis during germination (Parvaiz and Satyawati, 2008).

Tolerance of the plants to saline stress generally varies according to their development stages (Zaghdoudi et al., 2015). It has been reported by different researchers that salinity reduces water intake in seeds, inhibits germination by suppressing genes encoding GA biosynthetic enzymes, and also reduces germination rate due to stimulating ABA accumulated in tissues (Doğan and Budaklı Çarpıcı, 2016; Kim and Park, 2008; Li et al., 2016). In our study, increasing salt rates significantly decreased germination rates of fig genotypes. However, germinated seeds continued to grow on the same saline media for approximately 30 days. The response of seedlings to saline stress varies according to the saline levels and in general, high saline levels have decreased plant growth significantly. Halted or impaired plant growth in high saline stress stems from, in particular: ion toxicity, nutrient constraints, osmotic and oxidative stress. These mechanisms lead to disruption in the membrane lipids, proteins, and nucleic acids via the inflated levels of reactive oxygen species. Sodium toxicity is the main stress factor in the high saline lands by causing an increased rate of accumulation of reactive oxygen species (Mudgal et al., 2010). In our study, we found that the size of the seedlings in six cultivars was reduced by approximately 80% at 171.1 mM NaCl concentration compared to no saline control. Bursa Siyahı was the least susceptible to saline stress in the size of the seedlings reduction (75.9%). In all cultivars, germination can take place at 85.5 mM NaCl dose, while the Bursa Siyahı and Sultan Selim cultivars are less affected by saline stress than other fig cultivars with respect to the size of the seedlings. Saline stress leads to a 45% decline in the size of the seedlings in Bursa Siyahı and Sultan Selim compared to no saline control. In other cultivars, this reduction ratio reached up to 70%. Another crucial finding in our study is that the root length in the Bursa Siyahı and Sultan Selim cultivars did not curtail compared to the control at the 85.5 mM NaCl concentration. In terms of root development, the 85.5 mM NaCl dose did not pose any threat in these cultivars. Therefore, Bursa Siyahı and Sultan Selim are defined as saline stress-tolerant fig cultivars. However, in the Sarılop and Yeşilgüz cultivars we have reported as sensitive to salt, root growth at the dose of 85 mM NaCl decreased by 60% compared to the no saline control. Saline stress tolerant plant species underwent certain adaptation to manage this stressful condition. A boost in the root/canopy ratio, changes in chlorophyll content, adjustments in the leaf anatomy contributes to hinder leaf ion toxicity. Hence, water homeostasis is maintained by these adaptations and photosynthesis can be sustained without any hindrance (Acosta-Motos et al., 2017). Our findings are partially compatible with the findings of Emek (2018), who investigated the response of Bursa Siyahı to saline stress in vitro. Emek (2018), in his study, evaluated the effects of 0, 40, 80, 120, 240, 320 and 480 mM NaCl concentrations on the plant growth in Bursa Siyahı after 6-week incubation. According to this research, plants were healthy at 0-80 mM NaCl concentrations, browning at 120-240 mM NaCl concentrations and chlorosis and deaths were detected at 320-480 mM concentrations. The researcher stated that the observations in Bursa Siyahı were done up to 120 mM NaCl. In our study, observations were made in 6 fig cultivars up to 171.1 mM NaCl concentration. However, in this study, we recommend the use of the
dose of 85.5 mM NaCl, which is more reliable in evaluating the response of many fig genotypes to saline stress as a result of plant growth, because some plants do not survive after exceeding this concentration. Abdolinejad and Shekafandeh (2014) investigated the effects of different saline concentrations (0, 80, 120 and 160 mM) in in vitro conditions on two Persian fig cultivars: Anjir Sabz and Shah Anjir. At 160 mM NaCl concentration, Anjir Sabz reported having more chloride ion accumulation. Soliman and Alhady (2017) revealed that in their in vitro replication study with five local fig cultivars, a five-week salt application resulted in a significant reduction in plant growth and root length. In another study conducted with Brown Turkey and Royal cultivars of fig, salt was applied to irrigation water (Alswalmeh et al., 2015). This study announced that the Brown Turkey variant was less affected by saline stress than the Royal variant. In another in vitro replication study on Black Mission, Brown Turkey and Brunswick fig cultivars, different concentrations of NaCl were applied to replicating media containing different plant growth regulators. In the study, Black Mission, Brunswick, and Brown Turkey are listed as the most resistant cultivars to saline stress in Saudi Arabia respectively (Metwali et al., 2014). The effects of seven NaCl doses ranging from 1000-7000 mg L\(^{-1}\) (17.1-119.7 mM) on plant growth in four fig cultivars were tested in vitro (Yehia et al., 2018). Researchers suggested that 7000 mg L\(^{-1}\) (119.7 mM) NaCl is lethal. However, six fig genotypes showed development at 171.1 mM NaCl concentration in our study. The number of leaves of fig genotypes was recorded between 3.1 and 5.0 in 85.5 mM NaCl concentration. Yehia et al. (2018), reported that the number of leaves ranges between 2.4-4.0 at 5000 mg L\(^{-1}\) (85.5 mM) NaCl in the six-week-old plants. These values are consistent with our findings. In another study, saline stress resistance of four fig genotypes was tested by addition of 0.6, 4, 6, 8 dS m\(^{-1}\) saline containing water (6, 40, 60, 80 mM). Researchers found a negative correlation between increasing saline concentration and plant development. Of note, SxK genotype was determined as the most tolerant variant in their study (Zarei et al., 2016).

**Conclusion**

In this study, the growth performance of eight Turkish figs cultivars against saline stress was compared successfully in vitro. At 0 nM, 85.5 nM, 171.1 nM and 342.5 mM NaCl concentrations and 45 days after seed germination rate (%), seedling height (mm), number of leaves (pieces/seedling rootstock) and root length (mm) were assessed. The tolerance against saline stress differences between fig genotypes could be determined at highest 171.1 mM NaCl concentration regarding germination of seeds occur in vitro. In the study, the highest NaCl dose in which fig genotypes can be evaluated according to seedling development criteria was determined as 85.5 mM. A higher NaCl dose (171.1 mM), there were no germination in Sarılop and Yeşilgüz genotypes. When the germination rates and seedling growth were evaluated, it was revealed that Sultan Selim and Bursa Siyahı fig genotypes were more tolerant to saline stress than others. Sarılop, which is an important fig genotype in a dried fig market, was assigned as the most susceptible fig genotype to saline stress.

Most effective solution against prevailing salinity problem in agricultural areas is the development of tolerant cultivars and rootstocks to saline stress. Ratio of seed germination, which is important for hybridization and seedling production in fruit species, must be determined to the select genotypes under stress conditions in breeding
programs. Turkey is one of the important gene centers of the fig so it has large genetic variability for producing new fig cultivars tolerance to saline conditions by plant breeders of this country. In fig, this is the first study reporting in vitro germination performance of the seeds in salt conditions although some studies exist on the development of seedlings under salinity stress. It can be mentioned that a guiding and useful study has been presented for future fig breeding programmes.

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