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Evaluation of Salinity Tolerance Level of Some Pepper (*Capsicum annuum* L.) Cultivars

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Abstract **– Pepper, which is grown worldwide, has a 1.5 dS/m threshold value for soil salinity (ECe) and 1.0 dS/m threshold value for irrigation water salinity (ECw). Salinity tolerance of some landrace pepper cultivars, Demre Sivrisi, Çalabi İri Yağlık, Yalova Çarliston 341 and Kandil Dolma, were analyzed. Through the purpose, 0, 50, 100, 250 mM NaCl applications were held on germination percentage, relative growth rate, total chlorophyll content and lipid peroxidation criteria, relative water content, osmotic potential were considered. Results show that, all cultivars were effected negatively by the salt stress; the more salt stress was observed, the more negative impact took place. However, among the cultivars being examined through the study, Demre Sivrisi cultivar had relatively higher tolerance level than other cultivars and therefore can be recommended for pepper salt tolerance breeding studies.**

Keywords **– *Capsicum annuum* L, NaCl, Salt Tolerance, Pepper, *in vitro*.**

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I. Introduction

One of the most important abiotic stresses is salinity in soil or water that reduces plant growth and productivity worldwide. It is reported that more than 6% of land area of the world is affected by salinity stress. The main causes of salinity problem in agricultural areas are wrong management of soil and water, low rainfall and high evaporation. The technical processes (such as rehabilitation of soil, new irrigation methods) applied for salinity control is often expensive and create only a temporary solution [1].

Salinity is one of the most important environmental factors that influence plant growth and development [2,3]. The homeostatic balance of water potential and ion distribution within a plant is disrupted by high salt stress. Under salt conditions, sodium toxicity may lead to a number of disorders which affects germination, development, photosynthesis, protein synthesis, lipid metabolism, leaf chlorosis, and senescence. Plants have developed biochemical and molecular strategies to cope with the negative effects of salinity [4].

The conventional breeding programs play vital role in crop improvement. The efforts of plant breeding programs are to develop tolerant cultivars for the salinity fields and the identification of salt tolerant genetic resources through screening. Genetic diversity provides a practical means for screening and breeding for improved salt tolerant cultivars within a crop species [1].

Screening for salt tolerance and conventional breeding are difficult because of non-availability of uniformly salt affected fields as well as being time consuming and labor intensive [5]. Because of that, it is difficult to develop and screening tolerant varieties by using classic breeding methods [6]. In that sense, *in vitro* selection is a useful method to determine tolerant plants and tolerance level. As well as saving time and labor.

The magnitude of salinity effect depends on plant species and variety and level of salinity [7]. This study was conducted to determine the effective, practical, permanent and repeatable selection method for salinity toleration of pepper. To serve the purpose physiological and biochemical features of some landrace pepper cultivars were analyzed. Changes on chlorophyll amount and MDA amount in leaves of pepper cultivars under salt stress, which indicate the effects of salinity stress throughout vegetative growth on relative growth rate (RGR), leaf relative water content (RWC), leaf osmotic potential and cell membrane damage were observed and recorded.

It is supposed that the landrace pepper cultivars could be the perfect starting material for breeding salt tolerance cultivars. Therefore, the main aim of this study was to determine tolerance level of some landrace pepper cultivars and to generate a reference for the future breeding studies.

II. Materials and Methods

A. Plant Material

The seeds of landrace pepper cultivars, Demre Sivrisi, Calabi İri Yağlık, Yalova Carliston 341 and Kandil Dolma, were used in this study. Seed were surface sterilized with 10% sodium hypochlorite (NaClO) for 15 minutes, prior to three washes with sterile distilled water in laminar flow cabinet.

B. Experimental Design

Two different experiments were set up as down stated.

Experiment 1:

The seed germination capacities of landrace pepper cultivars were investigated in *in vitro* conditions at 24°C in the 16 h light/8 h dark regime. To serve purpose, seeds of each pepper cultivar were placed on petri dishes, containing basal Murashige and Skoog medium (MS) [8] combined with 0, 50, 100 and 250mM NaCl concentrations, 3% sucrose and 0.8% agar, pH 5.8. The experiment for each NaCl concentration was consisted of ten replications/cultivar (one petri dish represent one replication) and each replication contained 10 seeds/petri dish. The final percentage of seeds germination was recorded after 3 weeks culturing.

Experiment 2:

Seeds were placed on magenta containing thirty milliliters of basal MS medium and incubated at 24°C in the 16 h light/8 h dark regime. The basal medium

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consisted of MS medium, 3% sucrose and 0.8% agar, pH 5.8. The experiment for each genotype was consisted of ten replications (one magenta represent one replication) and for each replication contained 5 seeds/magenta.

After *in vitro* seed germination, the 12 days-old seedlings were transferred to MS medium supplemented with concentrations of 0, 50, 100 and 250 mM NaCl, 3% sucrose and 0.8% agar, pH 5.8, for stress treatments. Seedlings on the 5th day of treatment were harvested and stored at −20°C during analysis. Fully expanded youngest leaves were used for analysis.

C. Growth Analysis

Randomly chosen ten plants from experiment 2 were used for the growth analyses as they were separated to shoot and root fractions on the 5th day of NaCl treatments. Fresh weights (FW) of seedlings were measured to calculate the relative growth rate (RGR) of seedlings according to using the formula given by reference [9].

D. Leaf Relative Water Content (RWC)

Five youngest and fully expanded leaves were obtained from seedlings of each pepper cultivar and their FW was measured. The leaves were floated on deionized water for 6h under low irradiance. The turgid tissue was blotted to remove excess water and their turgid weights (TW) were observed. Leaves were dried in the oven in order to determine dry weight (DW). The relative water content (RWC) was calculated according to down stated formula [10].

$$RWC(\%) = [(FW - DW)/(TW - DW)] \times 100$$

E. Leaf Osmotic Potential

Vapor pressure osmometer was used to measure leaf osmotic potential. The data for leave osmotic potential were collected from six sample leaves per replicate. The results were converted to megapascal (MPa) according to reference [11] by multiplying with coefficient of 2.408×10^{-3}.

F. Total Chlorophyll Content

Leaves were collected to determine the chlorophyll content (chl*a*, chl*b*, chl*a*+*b*) and chlorophyll pigments were extracted by grinding leaves in 80% acetone in the dark at room temperature and were expressed as mg/gfm from equations of reference [12]. Absorbance of chlorophyll content was calculated between 470-663nm. The data were collected from 3 samples per replicate.

G. Lipid Peroxidation

The level of lipid peroxidation was determined according to the method described by reference [13] in terms of thiobarbutiric acid reactive substances (TBARS) content. TBARS were measured from the at 532nm absorbance and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. An extinction coefficient of 155 mM^{-1} cm^{-1} was used to calculate the concentration of TBARS.

H. Statistical Analysis

Variance analysis was applied for the obtained data using SPSS 10.0 program.

III. Results and Disscussion

A. Germination Percantage

In the first stage of the study, germination percentage of pepper seeds was investigated under different salt stress conditions. Final salt tolerance effect on germination percentage of the pepper cultivars are shown in Figure 1. Seeds of all pepper cultivars had 100% seed germination under control conditions. Under 50 mM and 100 mM NaCl, unlike Calabi İri Yağlık and Kandil Dolma, Demre Sivrisi and Yalova Carliston 341 cultivars were observed to have a higher germination percentage. In other words, germination percentage of Demre Sivrisi and Yalova Carliston 341 cultivars were not affected while Calabi İri Yağlık and Kandil Dolma cultivars had a decrease on seed germination under 50mM and 100mM NaCl treatments. The highest concenration (250mM NaCl) inhibited seed germination in all cultivars. Germination percentage of *Capsicum annuum* L. was known to be directly affected by stress conditions [14]. On the other hand, reference [15] reported that defining the proper harvest period would increase the germination percentage under salinity and osmotic stress conditions. In another study, seeds of *Capsicum annuum* L., *Solanum lycopersicum*, *Brasica oleracea* L. and *Sinapis alba* L. cultivars were subjected to GA3 application and exposed to salinity stress. Seed germination was only observed on *Capsicum annuum* L. cultivar under high salinity stress (800 mM) conditions [16]. As seen in these studies, use of plant growing stimulators can enhance germination percentage under salinity stress conditions but the defining main factor of germination is genotype and it is quite important to define highly tolerated genotypes for both breeding and cultivation studies.

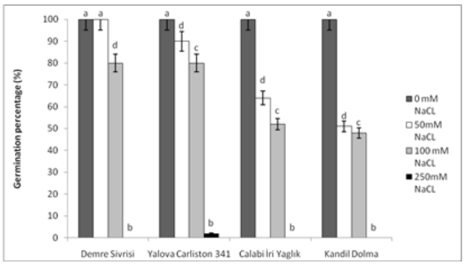

Fig. 1. Salt stress effects on germination percentage (%) in seedlings of pepper cultivars. Different letters represent statistically significant differences ($p < 0.05$) and vertical bars indicate ±SE.

B. Relative Growth Rate

The relative growth rate of all genotypes was reduced by salt stress treatments (Table 1). Çalabi İri Yağlık seedlings treated with 50 mM NaCl had a decreased on RGR by 19% while the greatest decrease at 250 mM NaCl treatment was observed in Kandil Dolma cultivars by 66%. RGR showed %38 and %39 decrease at 100 mM NaCl for Demre Sivrisi and Çalabi İri Yaglık, respectively. 50 mM NaCl treated Demre Sivrisi seedlings had the lowest RGR (%3). Stress conditions might have negative impact on plants RGR [17]. However, it is reported that, RGR values are more beneficial than

Table 1. Effects of salt stress on relative growth rate (RGR; mg mg-1 d-1) in seedlings of pepper genotypes.

RGR	Control	50 mM NaCl	100 mM NaCl	250 mM NaCl
Demre Sivrisi	0.3268 ± 0.0048	0.3157±0.0030	0.2015±0.0060	0.1826±0.0052
Yalova Carliston 341	0.3031±0.00042	0.2722±0.0048	0.2594±0.0055	0.1824±0.0016
Calabi İri Yağlık	0.4522±0.0026	0.3647±0.0015	0.2764±0.0037	0.1824±0.0021
Kandil Dolma	0.2771±0.0020	0.2398±0.00014	0.2427±0.0051	0094±0.0039

Table 2. Effects of salt stress on relative water content (RWC; %) in seedlings of pepper genotypes.

RWC	Control	50 mM NaCl	100 mM NaCl	250 mM NaCl
Demre Sivrisi	77.49±0.8895	74.60±1.06	52.73±0.4615	50.02±2.36
Yalova Carliston 341	87.48±2.5052	67.74±0.1844	64.99±1.3492	49.04±0.1815
Calabi İri Yağlık	83.00±2.505	58.06±0.218	56.71±0.218	49.18±1.018
Kandil Dolma	84.48±2.12	74.87±2.42	67.44±0.18	42.53±1.0183

absolute grow rate measurements in terms of plant growth comparison in salinity stress studies [18]. Reference [19] reported that all salinity stressed pepper cultivars had a dramatic decrease at their RGW amounts in 6 dSm^{-1} concentration and plants' reaction to salinity showed variation depending on genotype. Similar results were also reported by reference [20].

C. Relative Water Content

RWC is commonly used for determination of water balance in plants since it can be easily observed to see effects of osmatic stress. Previous studies showed that RWC of plants under salt stress got reduced [21, 22, 23, 24]. Present research results are in agreement with the previous findings since RWC of all cultivars used in the research decreased in accordance with salt levels. Salt stress decreased the RWC of all cultivars and the effect became obvious with the increase on salinity level (Table 2).

The lowest RWC decrease took place in Demre Sivrisi under 50 mM NaCl treated. RWC of Demre Sivrisi, Yalova Carliston 341 and Calabi İri Yaglık was decreased at 100 mM NaCl. The greater reduction in the RWC of all cultivars was observed at 250 mM NaCl treatment. The highest decrease RWC was observed in Kandil Dolma and the lowest decrease RWC was observed in Demre Sivrisi. Compared to all the genotypes and salt treatments, the lowest decrease on relative water content was obtained in Demre Sivrisi. The alteration of RWC levels might be an evidence of increase in membrane permeability.

D. Leaf Osmotic Potential

Plants water potential gets reduced by the increase on soluble salts in soil. The reduce on water potential can be balanced by the decrease on osmotic potential. Plants show resistance against the decrease on water dispensability and stresses by changing the water uptake system. Plants accumulate various compounds for avoiding low water potential and synthesis some proteins for repairing or preventing the damage of water loss in cells [25,26]. It is thought that osmotic arrangement due to osmotic stress may cause the decrease on osmotic potential [27]. The highest increase on osmotic potential was recorded in 250 mM NaCl treatment in a comparison among salt concentrations in all cultivars. On the other hand, no statistically significantly differences was observed among the cultivars subjected to same level salt concentration.

Osmotic potential of the all cultivars were decreased by salinity stress application (Figure 2). Under 50mM NaCl conditions, Demre Sivrisi, had the highest decrease with 25%. Osmotic potential of all cultivars under 250 mM NaCl stress application more adversely affected than plants under 50 and 100 mM NaCl stress application.

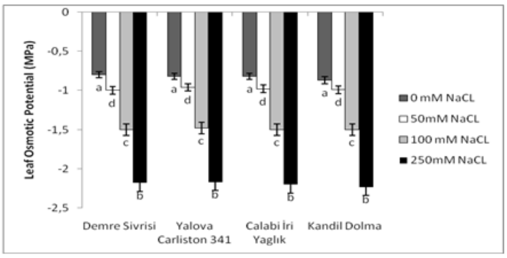

Fig. 2. Effects of salt stress on osmotic potential (MPa) in seedlings of pepper cultivars. Different letters represent statistically significant differences ($p < 0.05$) and vertical bars indicate ±SE.

E. Total Chlorophyll Content

To investigate the effect of salt stress on the chlorophyll content in four pepper genotypes, increase of NaCl concentration is accompanied by a decline of chlorophyll content (Figure 3). In the control conditions, the lowest chlorophyll content was observed in Yalova Carliston 341. Among the four cultivars subjected to 50 mM NaCl, the lowest chlorophyll loss was recorded for Kandil Dolma (%1). Chlorophyll content of Demre Sivrisi was identical at 250 mM NaCI and 100 mM NaCl treated groups. After the 250 mM NaCl treatment, the maximum chlorophyll loss was observed in Calabi İri Yaglık (%78).

Chlorophyll content in leaves can be affected negatively or positively by salt and osmotic stress. Reference [28] reported that tolerant with varieties had less chlorophyll degradation than sensitive ones. Changes on chlorophyll content may be related with genotypes, stress period and intensity of stress. It was reported that increase on chlorophyll degradation or the decrease on synthesis of chlorophyll might cause reduction on chlorophyll content [29]. Total chlorophyll amount in leaves tissue generally falls down under salinity stress. Findings of present research are in agreement with previous findings reported on chlorophyll contents of plants under salt stress. In present study, it was observed that chlorophyll contents in leaves decreased after different salt applications in all genotypes.

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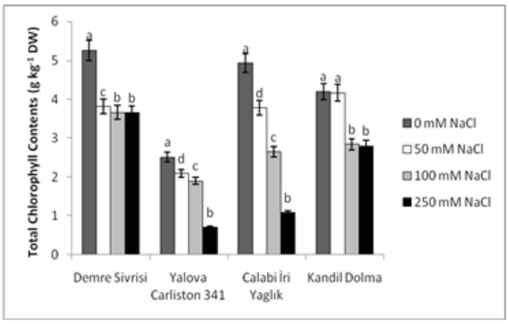

Fig. 3. The effects of salinity stress on total chlorophyll content (g kg-1 DW) in seedlings of pepper genotypes. Different letters represent statistically significant differences ($p < 0.05$) and vertical bars indicate ±SE.

F. Lipid Peroxidation

Lipid peroxidation level on membranes is an effective indicator of oxidative damage caused by salt and osmotic stress [30]. Lipid peroxidation, causing damages on cell membrane, produces malondialdehit (MDA). Another consequence of oxidative stress due to salt is the increase on MDA in cells. Findings on present study are in agreement with previous studies [31] since the MDA contents of all pepper cultivars increased with high salt concentration under stress condition.

Under the control conditions, MDA content of all genotypes were at the same level. MDA content of Demre Sivrisi cultivar under 50 mM NaCl treatment was 41% more than control group. MDA contents of 100 mM NaCl treated plants of Demre Sivrisi, Yalova Carliston 341, Calabi İri Yaglık, Kandil Dolma cultivars, as compared to their control groups, increased by 47%, 26%, 36% and 79%, respectively. Under the 250 mM NaCl treatment, MDA content most affected by Kandil Dolma genotype (96%). Under the effect of 250 mM NaCl treatment MDA content increased significantly during the experimental period in Demre Sivrisi, Calabi İri Yaglık and Kandil Dolma cultivars as compared to control groups, except cultivar Yalova Carliston. As shown in Figure 4, all genotypes analyzed, Kandil Dolma was heavily affected by salt stress.

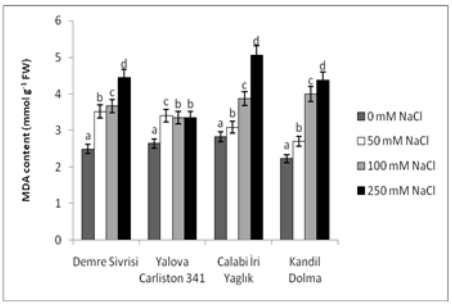

Fig. 4. The effects of salinity stress on lipid peroxidation in seedlings of pepper genotypes. Different letters represent statistically significant differences ($P < 0.05$) and vertical bars indicate ±SE.

IV. Conclusion

The present study was conducted to determine salinity tolerance rate of some landrace pepper cultivars which are thought to have potential to be used as breeding material. Experimental results revealed that Demre Sivrisi cultivars had higher level of salt toleration than other cultivars used and therefore can be recommended for salt tolerance breeding studies in pepper. Another important outcome of the present study is the fact that conducting salt tolerance studies in *in vitro* conditions shorten the duration of the study and can be recommended for further stress related studies.

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