

Full Length Research Paper

Genotypic variation in growth and physiological responses of common bean (*Phaseolus vulgaris* L.) seedlings to flooding

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Tolerance to flooding stress in root regions of some common bean genotypes (Beyaz Fasulye, Boncuk Sırık, Kökez, Oturak and Sırık) was investigated in terms of morphological and physiological. Plants were grown in a plant growth chamber at 26/18°C (day/night) temperature with RH 70% and 450 m⁻² s⁻¹ light intensity. Seedlings were exposed to flooding stress for 3 days when the plants had developed 3 to 4 true leaves. The results obtained showed that root dry weight and leaf area were reduced significantly by flooding treatment. The changes in leaf area showed differences between genotypes. It was found that flooding treatment did not affect the leaf relative water content (RWC) value. Flooding treatment decreased total chlorophyll content significantly. It was observed that some increases and decreases in the total sugar and lipid peroxidation (MDA) contents in root and leaf parts depend on genotypes and treatment. Beside that cell membrane injury and influence of flooding for each genotype were determined measuring the electrical conductivity. It was determined that the tolerance to flooding of five evaluated common bean genotypes, were change depending on root and leaf part. The results also showed that different genotypes responded differently to excess water in the soil, which could be linked to variation in growth and physiological responses. According to the evaluation, these results possibly suggest that 'Boncuk Sırık' was relatively tolerant genotype, whereas 'Sırık' and 'Kökez' were determined as more sensitive genotypes.

Key words: *Phaseolus vulgaris* L., common bean, excess water stress, flooding, lipid peroxidation, membrane injury, waterlogging.

INTRODUCTION

Flooding and submergence are major abiotic stresses and rank alongside water shortage, salinity and extreme temperatures as major determinants of species distribution worldwide (Visser et al., 2003; Jackson et al., 2009). Success or failure of crops in much arable

farmland can also be determined by the frequency and extend of flooding (Visser et al., 2003). Temporary and continuous flooding of soils are very common as a result of many factors including overflowing of rivers, storms, overirrigation, seepage from irrigation channels, inadequate drainage and impoundment of water by dams (Kozłowski, 1997; Mensah et al., 2006). Waterlogging of soil occurs not only in areas of heavy rainfall but also in arid regions where irrigation is practiced (Kozłowski, 1997).

Flooding imposes a severe selection pressure on plants principally because excess water in their surroundings can deprive them of certain basic needs notably of oxygen and of carbondioxide for photosynthesis (Jackson et al., 2009). Plant responses to flooding include, reduced stem growth, inhibition of leaf elongation, epinasty,

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Abbreviations: DMF, Dimethyle formamide; DW, dry weight; FW, fresh weight; H₂O₂, hydrogen peroxide; MDA, malondialdehyde; O₂⁻, superoxide; ¹O₂, singlet oxygen; OH, hydroxyl radicals; ROS, reactive oxygen species; RWC, relative water content; TBA, thiobarbituric acid; TCA, trichloroacetic acid.

chlorosis, reduced rates of CO₂ assimilation, reduced nutrient uptake and reduced root and shoot growth, plus formation of aerenchyma and adventitious roots, wilting and an increased susceptibility to attack by pathogens and predators (Bradford and Dilley, 1978; Aloni and Rosenshtein, 1982; Bradford and Hsiao, 1982). On the other hand, flood-tolerant plants survive waterlogging by complexes interactions of morphological, anatomical and physiological adaptations (Kozłowski, 1997).

One of the biochemical changes occurring when plants are subjected to waterlogging stress is the production of reactive oxygen species (ROS), such as superoxide (O₂⁻), singlet oxygen (¹O₂), hydrogen peroxide (H₂O₂) and hydroxyl radicals (·OH) (Subbubiah and Sachs, 2003; Jackson and Colmer, 2005). These ROS are highly reactive and can alter normal cellular metabolism through oxidative damage to lipids, proteins and nucleic acids (McKersie and Leshem, 1994). Malondialdehyde (MDA) content, a product of lipid peroxidation, has been considered an indicator of oxidative damage (Tang-Bin et al., 2010).

On the other hand, flood tolerance varies greatly among plant species, genotypes and rootstocks and is influenced by plant age, time and duration of flooding, condition of the floodwater and site characteristics (Kozłowski, 1997).

Common bean (*Phaseolus vulgaris* L.) is a significant source of dietary protein in many developing countries (Duranti and Gius, 1997) and it is one of the most important vegetable crops grown in Turkey. Common bean is known as a sensitive plant to water-stress condition (Latdawan, 1993; Singer et al., 1996). Problems caused by flooding may be solved by growing flood-tolerant crops (Yetisir et al., 2006). Therefore, attempts have been made to breed for increased flooding tolerance and modify crop cultivation or management practices and avoid flooding injury (Lin et al., 2008).

Therefore, the objective of this study was to investigate the morphologic and physiological changes in five common bean genotypes during short-term flooding treatments and their role in flooding tolerance.

MATERIALS AND METHODS

Plant material and growth conditions

In order to determine the genotypic differences during 3 days-flooding stress, five common bean genotypes were used (Table 1). Seeds of the common bean genotypes were sown into viols (31.5 x 51.5 cm) filled with a mixture of peat, perlite and vermiculite (2:1:1). Plants were grown in a plant growth chamber (DAIHAN WGC-1000, South Korea) at 26/18°C (day/night) temperature, with relative humidity 70% and 450 μmol m⁻² s⁻¹ light intensity (Khadri et al., 2006). At early seedling stage (2-week-old), seedlings were flooding by placing the viols in a container. Seedlings were submerged to the level of soil surface for 3 days in the container.

Table 1. Local name and origin of the genotypes used in this study.

Local name	Origin
Beyaz Fasulye	Kırıkkale
Boncuk Sırık	Artvin, Yusufeli
Kökez	Çukurova
Oturak	Eskişehir, Muttalip
Sırık	Artvin, Yusufeli

Growth measurement

For growth measurements, three plants per replication were sampled at the end of the experiment. Plants were separated into leaf and root parts and the plant material was dried at 70°C for 48 h and then weighted for dry weight (DW). The leaf area was recorded by using a digital leaf meter (LI-3000 portable area meter produced by LICOR Lincoln, Nebraska, USA).

Leaf relative water content (RWC) and total chlorophyll content

For leaf relative water content (RWC, %) and total chlorophyll content measurements, three plants per replication were sampled at the end of the experiment. RWC was measured as follows; RWC = [(fresh weight-oven dry weight)/ (turgid weight-oven dry weight)] X 100. For obtaining turgid weight, 1.5 cm leaf discs were floated on distilled water in a petri dish for 4 h at room temperature. After incubation, leaf discs were removed from the petri dish, surface-blotted and immediately weighed. For oven drying, leaf discs were put in a new dry petri dish with lid and placed in an oven at 70°C for 48 h. After incubation, leaf discs were weighed (Barr and Weatherley, 1962).

Total chlorophyll content was measured colorimetrically as described by Moran and Porath (1980). 1.5 cm 3 leaf discs were put in 20 ml bottle and add 5 ml dimethyl formamide (DMF) each bottle. Samples were kept at 4°C and at dark for 72 h. The absorbance were measured at λ= 652 nm (Perkin Elmer Lambda 25, USA). The content was calculated according the formula:

$$\text{Total chlorophyll (mg/ g FW)} = \text{O.D.652} \times 29 \times \text{dilution factor/ mg/g FW}$$

Total sugar

Total sugar were extracted by suspending 100 mg of samples (leaf and root) in 5 ml of 8:10 (v/v) ethanol in an 85°C water bath for 1 h after which the liquid was removed from the tissue. This procedure was repeated four-times for 1 h, 30, 15 and 15 min, respectively. The ethanol solutions (approx. 20 ml) were combined and evaporated to dryness at 55°C with the aid of continuous ventilation. Pellets were dissolved in 1 ml of distilled water. Total sugar contents were determined using the anthrone reagent method (Van Handel, 1968) in a spectrophotometer (Perkin Elmer Lambda 25, USA) at 620 nm, with glucose solutions as standards.

Lipid peroxidation (malondialdehyde=MDA content)

MDA is a final decomposition product of lipid peroxidation and has been used as an index for the status of lipid peroxidation. MDA

Table 2. The effects of flooding treatment on growth parameters in plants of five common bean genotypes, when plants were subjected for 3 days to flooding treatment.

Genotype	Treatment	Leaf DW (g)	Root DW (g)	Leaf area (cm ²)
Beyaz Fasulye	Control	0.074	0.141	39.39
	Flooding	0.066	0.107	32.48
Boncuk Sırık	Control	0.073	0.053	36.25
	Flooding	0.077	0.049	37.93
Kökez	Control	0.082	0.055	42.87
	Flooding	0.068	0.045	36.97
Oturak	Control	0.142	0.185	49.50
	Flooding	0.149	0.166	39.24
Sırık	Control	0.104	0.093	49.84
	Flooding	0.112	0.088	46.96
ANOVA				
Genotype (A)		*	*	*
Treatment (B)		ns	*	*
AxB		ns	ns	ns

ns and * denote not significant and significant, respectively (P < 0.05).

content was estimated by in the roots and leaves according to method of Rajinder et al. (1981). A hundred milligram of samples (roots and leaves) was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10 000 × g for 5 min at 4°C. Aliquot of 0.3 ml supernatant was mixed with 1.2 ml of 0.5% thiobarbituric acid (TBA) prepared in TCA 20% and incubated at 95°C for 30 min. After stopping the reaction in an ice bath for 5 min, samples were centrifuged at 10 000 × g for 10 min at 25°C. The supernatant absorbance at 532 nm was then measured using a spectrophotometer (Perkin Elmer Lambda 25, USA). After subtracting the non-specific absorbance at 600 nm, MDA concentration was determined using the extinction coefficient 155 mM⁻¹ cm⁻¹.

Measurement of injury

Flooding injury of root and leaf parts of seedlings was determined by measuring electrolyte leakage as described by Arora et al. (1998) with some modifications. Briefly, root samples with 2 cm and leaf disks in 1.5 cm diameter were taken separately from each of five plants per treatment (control and flooding). They were lightly rinsed in distilled water, gently blotted with paper towel and placed in test tubes (one leaf disc or root piece per test tube). 20 ml of distilled water was added to test tubes which were then vacuum infiltrated to allow uniform diffusion of electrolytes. Tubes were shaken on a gyratory shaker (250 rpm) for 4 h at room temperature. Electrical conductivity of each sample was measured using conductivity meter (YSI 3200, USA). Electrical conductivity of each sample was measured once more after the tubes were autoclaved (124 kPa, 121°C, 20 min) and cooled. Percentage injury at flooding was calculated from ion leakage data using the equation (Arora et al., 1992):

$$\% \text{injury} = [(\%L_{(t)} - \%L_{(c)}) / (100 - \%L_{(c)})] \times 100$$

Where, %L_(t) and %L_(c) are percentage ion leakage data for the

treatment and control samples, respectively.

Statistical analysis

The experiment was arranged in a randomized block design with three replications. Data were tested by SPSS 13.0 for Windows program and mean separation was accomplished by Duncan test at P < 0.05.

RESULTS

Growth measurement

Leaf DW, root DW and leaf area were used to assess the adverse effect of flooding on plant growth. Growth responses of common bean genotypes to flooding treatments are shown in Table 2. When compared with control treatment, it was determined that flooding treatment did not influence leaf DW, but it decreased the root DW and leaf area in five common bean genotypes.

Although, changes in the leaf DW values were not significant statistically, leaf DW was decreased at 'Kökez' and 'Beyaz Fasulye' genotypes with 17% and 11%, respectively during flooding treatments. On the other hand, flooding treatment increased the leaf DW values in 'Sırık', 'Boncuk Sırık' and 'Oturak' genotypes. The root DW was affected by flooding treatment in all genotypes. The reductions were more pronounced for 'Beyaz Fasulye' and 'Kökez' genotypes. Compared with the control, treatment leaf area was reduced by 21% in 'Oturak' genotype, 18% in 'Beyaz Fasulye' genotype and

Table 3. The percentage of injury (based on electrolyte leakage) in the root parts and leaf discs of common bean genotypes, when plants were subjected for 3 days to flooding treatment.

Genotype	Injury (%) in the root part	Injury (%) in the leaf part
Beyaz Fasulye	7,22 ^c	12,90 ^a
Boncuk Sırık	14,16 ^{bc}	9,49 ^b
Kökez	38,75 ^a	1,62 ^d
Oturak	23,39 ^{ab}	4,53 ^c
Sırık	37,52 ^a	4,64 ^c

Values not associated with the same letter are significantly different ($P < 0.05$).

14% in 'Kökez' genotype at flooding treatment. However, leaf area value increased in 'Boncuk Sırık' genotype.

Leaf relative water content (RWC) and total chlorophyll content

Effect of flooding treatment on RWC and total chlorophyll content of five common bean genotypes are summarized in Figure 1 (A, B). RWC of leaves was maintained despite the flooding in five common bean genotypes. On the other hand, when compared with control treatment, flooding treatment caused a decrease in total chlorophyll content in all genotypes. The reduction was greater in 'Sırık' genotype with 28%.

Total sugar

Total sugar content in root and leaves of common bean genotypes at the end of flooding treatment is presented in Figure 2 (A, B). At the end of the experimental period, total sugar content in roots and leaves was not affected by flooding treatment. However, significant differences were determined between genotypes for sugar content in leaves. In results of measurements, the least sugar content in leaves was shown in 'Beyaz Fasulye' and 'Oturak' genotype, followed by 'Kökez' and 'Sırık'. The highest sugar content was determined in leaves of 'Boncuk Sırık' genotype (Figure 2B).

Lipid peroxidation (MDA)

In this study, as shown in Figure 2 (C, D), non-significant changes in MDA content of the root parts were recorded between control and flooding treatment. On the other hand, in the leaves MDA levels, control plants remained significantly high compared with flooding plants (Figure 2D). Beside that there were significant differences between genotypes for MDA content in roots and leaves. The content of MDA in roots was higher in 'Oturak' and

'Kökez' genotypes than in 'Boncuk Sırık', 'Beyaz Fasulye' and 'Sırık' genotypes (Figure 2C). In the leaves, MDA content of 'Oturak' genotype was highest, followed by that of 'Beyaz Fasulye' genotype. MDA content in leaves of 'Sırık', 'Kökez' and 'Boncuk Sırık' genotypes were lower than these (Figure 2D).

Cell membrane injury

The percentage of injury (based on electrolyte leakage) in root parts and leaf discs as a function of flooding was shown in Table 3. The data indicated that percentage injury changed depending on the genotypes in leaf and root parts. Regarding the effect of treatment, flooding caused the less percentage of injury in 'Beyaz Fasulye' and 'Boncuk Sırık' genotypes (7.22 and 14.6%, respectively) than 'Kökez', 'Sırık' and 'Oturak' genotypes in root tissues (Table 3). In leaf tissues, comparison of the percentage of the injury of five common bean genotypes, 'Beyaz Fasulye' and 'Boncuk Sırık' exhibited greater percentage of injury than 'Sırık', 'Oturak' and 'Kökez' with flooding treatment (Table 3).

DISCUSSION

Inhibition of growth observed in this study confirms earlier results (Singer et al., 1996) and is similar to that in watermelon plants (Yetisir et al., 2006), field bean (Pociecha et al., 2008) and tomato (Else et al., 2009). On the other hand, the leaves of the plants under flooding stress showed epinasty after 2 days of flooding, however, in the control treatment, most leaves looked green and healthy (data not shown).

At the physiological level, flooding could greatly affect plant water relations (Striker et al., 2007). Some studies on a variety of herbaceous and woody species however, suggest that the more common response to flooding is partial stomatal closure and the maintenance of high leaf water potential (Bradford and Hsiao, 1982; Bradford, 1983). In this study, the short-term flooding for 3 days

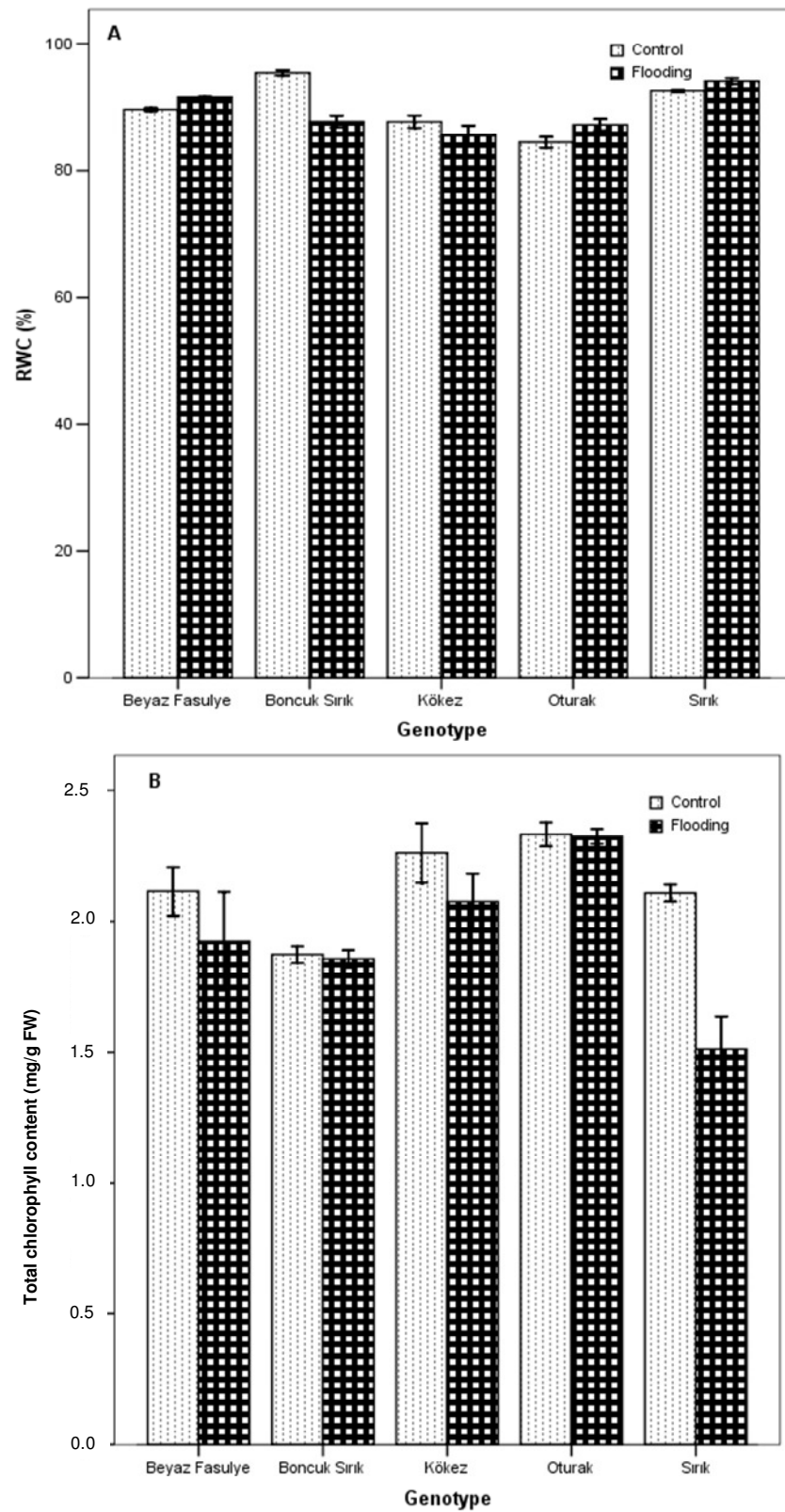


Figure 1. The effects of flooding treatment on leaf relative water content (RWC) (Panel A) and total chlorophyll content (Panel B) in common bean genotypes. Error bars represent \pm SE of three replications.

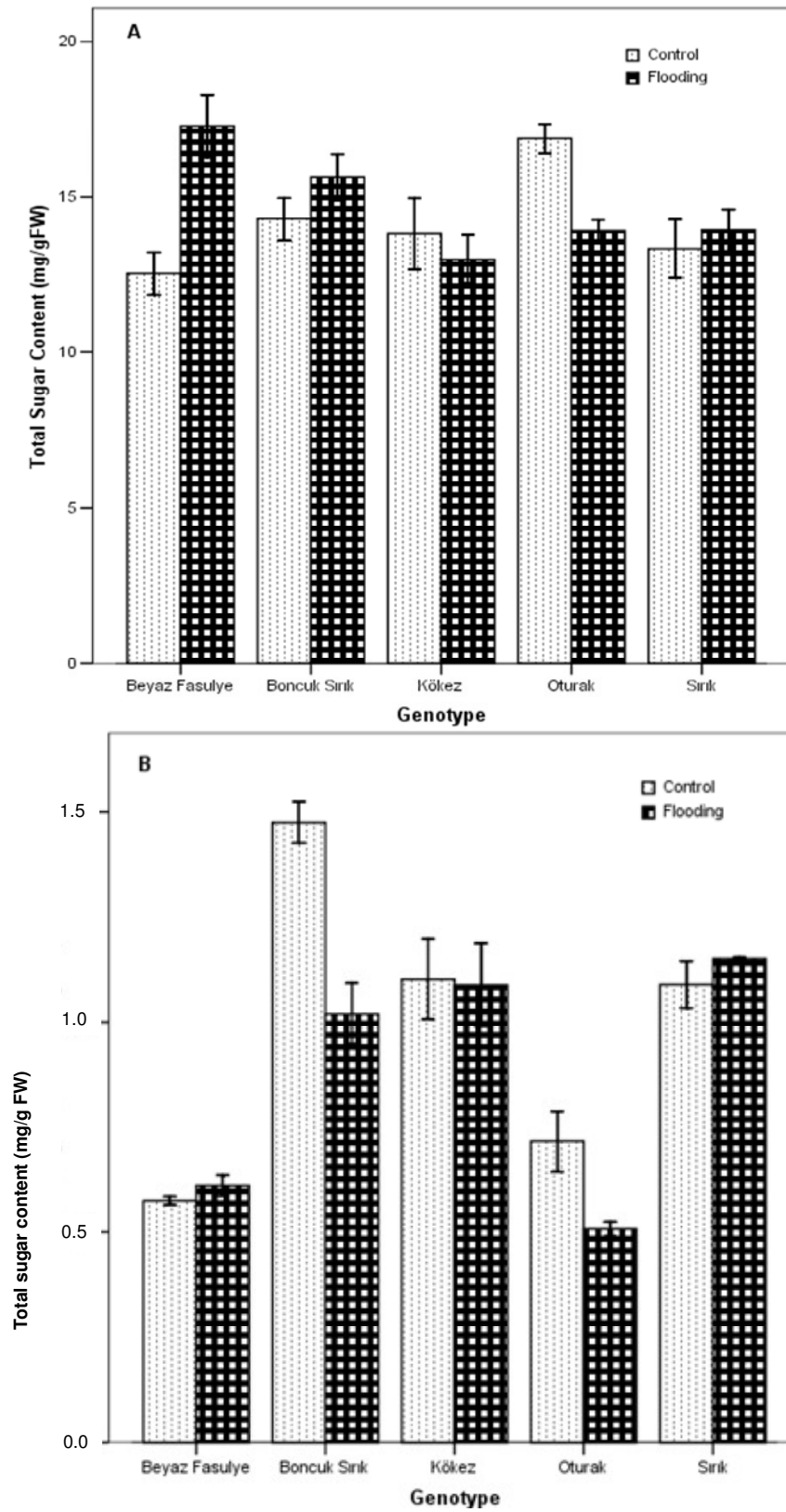


Figure 2. The effects of flooding treatment on total sugar content in the roots (Panel A); total sugar content in the leaves (Panel B)

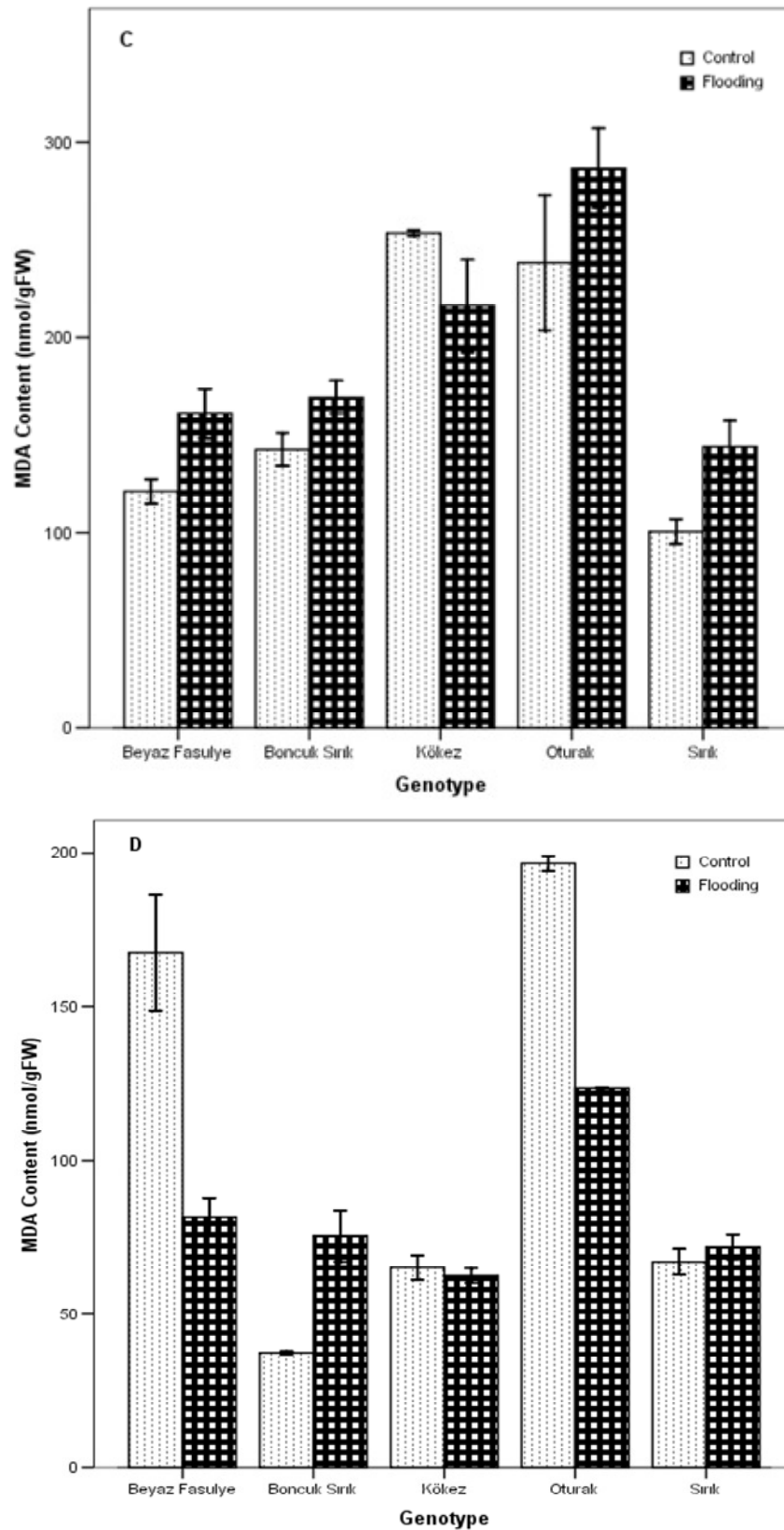


Figure 2 (continued) The effects of flooding treatment on MDA content in the roots (Panel C); MDA content in the leaves (Panel D)

was apparently insufficient to alter water relation parameters of five common bean genotypes (Figure 1A). Similar results were also obtained in citrus rootstock seedlings during short-term flooding period (Garcia-Sanchez et al., 2007).

One of the most important changes under stress is the decrease of the total chlorophyll content (Levitt, 1980). Flooding stress had a harmful effect on the common bean and reduced total chlorophyll content of the plant leaves during flooding (Figure 1B). Similar decrease of total chlorophyll content in leaves was observed in bean plants by Singer et al. (1996). Decreases of total chlorophyll content as a result of flooding was also observed in maize (Rao et al., 2002), in sesame (Mensah et al., 2006) and in onion (Yiu et al., 2008).

In this study, total sugar content of 3 days flooding plant was non-significantly different than control plant in roots and leaves (Figure 2A, B). In reality, there have been different reports about this subject in the literature. For instance, sugar content of leaves decreased with flooding treatment in *Cleopatra mandarin* (Garcia-Sanchez, 2007). However, waterlogging markedly increased glucose and sucrose in shoots and roots of *Vigna sinensis* and *Zea mays*, but greatly decreased poly-saccharides (Alla et al., 2001). Beside that in theory, if the translocation path is blocked, assimilates in leaves will not be able to reach the roots, thus, resulting in a sugar deficiency in the roots (Liao and Lin, 2001).

Lipid peroxidation is a natural metabolic process under normal aerobic conditions and it is one of the most investigated consequences of ROS action on membrane structure and function (Blokina et al., 2003). Lower levels of MDA indicate better oxidative stress tolerance. In this study, MDA content of 3 days flooding plant was lower (or non-significant different) than control plant (Figure 2C, D), indicating low cell damage in the flooding treatment. Similarly, in sweet potato, it was found that MDA content of 1-day flooded plant was lower (or non-significant different) than non-flooded plant (Lin et al., 2008). Similar results have also been obtained from Hossain et al. (2009) in citrus plants at the end of 18 days of flooding treatment.

Cell membrane stability has been widely used to express stress tolerance and higher membrane stability could be correlated with abiotic stress tolerance (Premachandra et al., 1992). On the other hand, when crop plants are subjected to soil waterlogging or an anaerobic condition, their root and shoot systems respond differently (Liao and Lin, 2001).

In our experiment, it was determined that the tolerance to flooding of five evaluated common bean genotypes, were change depending on root and leaf part. According to the general evaluation, these results possibly suggest that 'Boncuk Sırık' was relatively tolerant genotype, whereas 'Sırık' and 'Kökez' were determined as more sensitive genotypes. The results showed that different

genotypes responded differently to excess water in the soil, which could be linked to variation in growth and physiological responses. Nevertheless, additional measurements should be made in future studies at long-term flooding condition to obtain further information.

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