HEAT STRESS TOLERANCE OF SOME GREEN BEAN
(*Phaseolus vulgaris* L.) GENOTYPES

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Abstract

The seedlings of 3 green bean genotypes ('Balkız', 'Ferasetsiz', 'Local Genotype') were grown for 4 weeks at 30/15°C (day/night) temperature with 65% humidity and then transferred to a growth chamber. The temperature in the growth chamber was increased stepwise to 35, 40, 45 and 50°C to create a heat-stressed environment. In addition, to determine HST (LT_{50}), the leaf discs of each genotype were exposed to 35, 40, 45, 50, 55 and 60°C. According to results, 'Ferasetsiz' and 'Local Genotype' had the highest leaf relative water content (RWC), while the 'Balkız' had the lowest. However, 'Ferasetsiz' and 'Local Genotype' had the lowest loss of turgidity and malondialdehyde (MDA), while 'Balkız' had the highest. The leaf area and chlorophyll content (Chl) were not associated with the HST of genotypes. Many protein bands, in mass range 27–157 kDa, were detected depending on the genotypes and heat treatments. Besides, a few new proteins appeared in response to heat stress. Based on the data collected, 'Ferasetsiz' and 'Local Genotype' were determined to heat tolerant genotypes, while 'Balkız' was relatively heat sensitive genotype.

Key words: green bean, Phaseolus vulgaris L., high temperature, malondealdehyde, SDS-PAGE, stress tolerance.

INTRODUCTION

Higher growing period temperatures can have impressive affects on agricultural productivity, farm revenues, and food safety (Battisti & Naylor, 2009). According to the report of the Intergovernmental Panel on Climate Change (IPCC), 0.3-0.7°C of warming is expected globally between 2016 and 2035 and to 0.3-4.8°C by the end of this century (IPCC, 2014). Heat stress generally damages photosynthetic activity, and the reduced water content caused by heat has negative effects on cell division and growth (Hasanuzzaman et al., 2013). Heat stress also causes impaired to the proteins, lipid liquefaction or perturbation of membrane integrity (Levitt, 1980).

Plants have evolved delicate mechanisms to combat stressful environments. A gradual increase in temperature informs organisms that they should ready themselves in case they undergo even warmer conditions (Salomé, 2017). This process has significant effect on inducement of tolerance to lethal high temperatures (Hasanuzzaman et al., 2013). Legumes are valued worldwide as a primary food source and low-cost meat alternative and considered to important rotational crop used to improve soil nitrogen status (Maphosa & Jideani, 2017). Because of the growing request for plant products and environmental pressures on agro-eco systems, it comes out that legumes would play an important function in here after cropping systems (Stagnari et al., 2017). On the other hand, an increase in global temperature, as a result of climate change, increments the risk of a heat stress-induced diminution in legume crop yield (Ozga et al., 2017). Hence, to ameliorate heat tolerance in legume crops contributing to improved food security. In this research, genotypic variations in 3 green bean genotypes and their relationship to heat-stress tolerance were investigated. The main aim of the study is to provide data for afterwards, more comprehensive works on green bean heat-tolerance.

MATERIALS AND METHODS

The seeds of green bean genotypes, 'Balkız', 'Ferasetsiz', and 'Local Genotype' were sown in pots 14 cm × 12 cm containing a mixture of peat, perlite and soil (1: 1: 1). The plants were grown for 4 weeks (3-4 trifoliate leaves) in a

controlled greenhouse with day/night temperature of 30°C/15°C and approximately 65% relative humidity. The plants were watered as needed to avoid any water stress.

The plants were transferred to a growth chamber (DAIHAN WGC-1000, South Korea) with a relative humidity of 65%, a 16/8 h (light/dark) photoperiod regime and a 450 $\mu mol/m^2/s$ light intensity. Then, the temperature was increased stepwise, 5°C every 24 h from 35 to 40, 45 and 50°C to impose heat stress gradually. Plants were kept in the growth chamber for 24 h at each temperature. Control plants were held in the greenhouse during the treatment with a 30/15°C day/night temperature. All temperature treatments were replicated three times with all genotypes. Samples that were obtained at each treatment temperature were analyzed for leaf relative water content (RWC), loss of turgidity, leaf area, chlorophyll content (Chl), lipid peroxidation (MDA) and protein content.

Leaf RWC (%) and loss of turgidity were measured according to Gulen and Eris (2003). Briefly, 1.5 cm leaf discs were taken and the fresh weight (FW) was recorded. Then, the leaf discs were floated on distilled water in a petri dish for 4 h at room temperature then leaf discs were taken out from the petri dish, blotted and turgid weight (TW) was recorded. The leaf discs were oven-dried for 48 h at 70 °C for the dry weight (DW). The leaf RWC and loss of turgidity were calculated as follows:

RWC (%) = [(FW -TW)/(TW -DW)]*100 and Loss of turgidity (%) = [(TW-FW)/ TW]*100

The leaf area was determined for three plants per replicate by a portable area meter (LI-3000, Li-Cor, Lincoln, NE, USA).

Changes in Chl of green bean leaves were analyzed spectrophotometrically as described by Moran and Porath (1980). Leaf samples subjected to dimethylformamide (DMF) extraction were incubated at +4°C for 72 hours. The absorbance of supernatants was measured at 652 nm by a spectrophotometer (Perkin Elmer Lambda 25, USA). The content was calculated as mg/g FW.

Malondialdehyde content was determined in the leaves as described by Rajinder et al. (1981). Briefly, 100 milligram of leaf sample was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA) and centrifuged at 10000×g for 5 min at 4°C (Beckman Coulter Allegra 64R, USA). An aliquot supernatant (0.3 ml) 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 10000×g for 10 min at 25°C. The supernatant absorbance was read at 532 and 600 nm using a spectrophotometer (Perkin Elmer Lambda 25, USA). Malondialdehyde content was determined using the extinction coefficient 155 mM/cm.

To determine total soluble protein (TSP) content, fully expanded leaf material was collected from each plant group at each temperature application step. Triplicate samples of leaf tissues were immediately frozen and ground in liquid N_2 and stored at - 80 °C until used. Total soluble proteins were extracted in the light of Shen et al. (2003) method with few modifications. In brief, 0.25 g of each sample was homogenized in 1 ml homogenate buffer containing 25 mM Tris-base (pH 7.8), 275 mM sucrose, 2 mM EDTA, 10 mM Dithiothreitol (DTT), 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and 1% polyvinylpolypyrolidone (PVPP). The homogenate was transferred into an eppendorf tube and then centrifuged at 10000 rpm for 10 min at 4°C. Protein content was measured using the Bradford assay method (Bradford, 1976).

Discontinuous sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed with a PROTEAN tetra vertical electrophoresis unit (Bio-Rad, Hercules, CA, USA) using 0.04 stacking gel and 0.125 separating gel. An equal amount of total protein (7.5 mg) was loaded for each sample and gels were stained with Coomassie Brilliant Blue G-250.

Cell membrane injury and HST (LT_{50}; denoted by electrolyte leakage) were determined as previously described by Arora et al. (1998) to assess the six temperature steps-35, 40, 45, 50, 55, 60°C. Briefly, green bean leaf discs of 1.5 cm diameter from each treatment group were placed in test tubes containing 500 μl of deionized water. Tubes were capped and placed in a thermostatically controlled water bath then the temperature was increased by 5°C at 30 min intervals up to 45°C, and then the bath

temperature was increased by 1°C at 5 min intervals up to 60°C. Samples were allowed to equilibrate and then were kept for 30 min at each temperature (35, 40, 45, 50, 55 and 60°C). Three tubes per temperature were removed and placed in an incubator. Three tubes of the control (un-stressed) discs remained at 30°C as controls. One hour after the last sample was removed from the bath, 10 mL of deionized water was added to each test tube, and it was incubated overnight. The electrical conductivity of each solution was measured using a conductivity meter (YSI 3200, USA) then immediately autoclaved. Total conductivity was determined once more when the solution in test tubes cooled down to room temperature. Cell membrane injury was defined as the percentage of total ions present in the tissue (Gulen & Eris, 2003). In addition, HST (LT_{50}) was calculated as the mid-point between the maximum injury and the control, which expresses the temperature causing half-maximum percentage injury.

The experiment was arranged in a randomized block design, with three replications. The data were tested with SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA) and the means were evaluated by the Duncan test at P<0.05.

RESULTS AND DISCUSSIONS

The changes of leaf RWC and loss of turgidity values of the genotypes are shown in Figure 1A and Figure 1B, respectively. The data from the present study showed that leaf RWC slightly increased at temperatures up to 45°C in 'Ferasetsiz' and 'Local Genotype', while it decreased significantly at 45°C and 50°C. However, leaf RWC value was greater only at 35°C than that of control treatment and a remarkable decrease was observed at temperatures from 40 °C and up in 'Balkız' genotype (Figure 1A).

In contrast to RWC results, loss of turgidity of leaf samples slightly decreased at temperatures up to 45°C in 'Ferasetsiz' and 'Local Genotype', while it increased significantly at 45°C and 50°C. On the other hand, loss of turgidity value was lower only at 35°C than that of control treatment and it increased significantly at temperatures from 40°C and up in 'Balkız' genotype (Figure1B). A two-way

ANOVA revealed significant effects of temperature, genotype and the interaction between temperature and genotype on leaf RWC and loss of turgidity (Table 1).

Leaf RWC represents an indicator of plant water balance because it expresses the absolute water amount the plant requires to reach artificial full saturation (Siddiqui et al., 2015). A decrease in RWC in response to high temperature was also reported in strawberry (Gulen & Eris, 2003; Kesici et al., 2013), olive (Cansev, 2012) and lablab bean (Myrene & Devaraj, 2013). The differences between the leaf RWC in genotypes can be a sign of difference in leaf hydration, leaf water deficiency and physiological water conditions. First of all heat stress causes a decrease in leaf RWC and the loss of turgidity, which is a consequence of elevated transpiration (Cansev, 2012; Turhan et al., 2014). Besides, De Belie et al. (2000) pointed out that the cause of turgor loss in high temperature stress conditions may be related to the permeability of the cell membrane at high temperature.

Table 1. Results of analysis of variance (ANOVA) of genotype (G), temperature (T) and their interactions with RWC, Loss of turgidity, Leaf Area, Chl, MDA and TSP content in leaf tissues. Numbers represent F values relative to a significance level of 0.05

Dependent Variable	Independent Variable		
	G	T	GxT
RWC	9.054*	350.439*	16.422*
Loss of Turgidity	5.461*	188.007*	12.084*
Leaf Area	58.115*	60.589*	3.308*
Chl	2.216ns	74.421*	10.284*
MDA	257.973*	687.561*	62.923*
TSP	38.555*	25.543*	10.400*
LT_{50}	265.542*	1032.070*	45.495*

* Significant at p<0.05, ns Non-significant.

The changes in leaf area values of green bean genotypes are given in Figure 2A. Although there is no significant change in the leaf areas of genotypes up to 45°C, a remarkable change was observed when it comes from 45°C to 50°C. According to the average values, the maximum leaf area value occurred in 'Ferasetsiz' (42.09 cm^2) and 'Balkız' (37.86 cm^2), whereas the minimum leaf area value

occurred in 'Local Genotype' (27.69 cm^2). When the treatments were compared, the highest leaf area value was determined in the control treatment with 47.66 cm^2, while the lowest leaf area value was found at 50ºC treatment with an average of 23.09 cm^2 (Figure 2A). A two-way ANOVA revealed significant effects of temperature, genotype and the interaction between temperature and genotype on leaf area (Table 1).
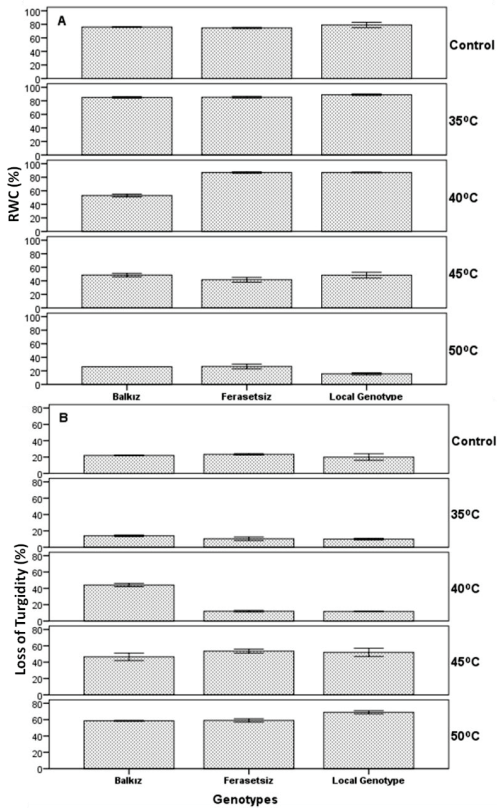

Figure 1. Leaf RWC (Panel A) and loss of turgidity (Panel B) of three green bean genotypes in heat-stressed and control samples. Vertical bars indicate ±S.E. of three replications

According to the results obtained from this study, it was determined that leaf areas of the genotypes generally decreased during high temperature applications. It was reported that the leaf area significantly reduced in sorghum (Djanaguiraman et al., 2010) and potato (Beetge & Krüger, 2019) depending on the high temperature applications. This situation may have thought to be because of the decrease in leaf area and decreases in photosynthetic activity parallel to the slowing of plant growth and development due to high temperature.

The changes in Chl in the green bean genotypes are shown in Figure 2B. According to the average values, the lowest Chl occurred in 'Balkız' (4.28 mg/g FW) and 'Ferasetsiz' (4.31 mg/g FW), whereas the highest Chl occurred in 'Local Genotype' (4.83 mg/g FW). In general, the average Chl in the control treatment (4.4 mg/g FW) was significantly lower than that in the 50 ºC treatment (8.35 mg/g FW). A two-way ANOVA revealed significant effects of temperature and the interaction between temperature and genotype on Chl but no significant effect of the genotypes (Table 1).

According to the results obtained from this study, it was determined that 50°C treatment increased total Chl in all genotypes. There are different results in the literature about this issue. For example, high temperature stress reduced the total Chl in mulberry (Chaitanya et al., 2001) and citrus (Guo et al., 2006). Besides, Cansev (2012) reported that high temperature applications did not affect total Chl up to 45°C in olives but decreased Chl from 50°C. Liu and Huang (2000) and Kesici et al. (2013) reported that a relationship between Chl and heat-tolerance in *Agrostis palustris* and strawberry, respectively. This can be explained by the fact that the physiological responses of plants to the stress conditions vary greatly depending on the species, cultivar, severity and the source of the stress.

The changes in MDA content in the leaf tissues are shown in Figure 3. The data from the five sampling treatments showed that 'Ferasetsiz' had the lowest MDA content with 46.8 nmol/g FW. 'Local Genotype' followed this genotype with 49.8 nmol/g FW. 'Balkız' had the highest MDA content with 90.3 nmol/ g FW. When the heat treatments were compared, the lowest MDA content was determined with an average of 24.7 nmol/g FW at 35ºC, while the highest MDA content was determined at 50ºC with 139.7 nmol/g FW. A two-way ANOVA revealed significant effects of temperature, genotype and the interaction between temperature and genotype on MDA content (Table 1).

Malondialdehyde is a final decomposition product of lipid peroxidation and is often used as an indicator for the status of lipid

peroxidation (Smirnoff, 1995). High temperature exposure produced increased MDA content in all genotypes (Figure 3). This result is consistent with previous findings in rice (DongGi et al., 2007) and strawberry (Kesici et al., 2013). Gaschler and Stockwell (2017) also reported that the increase in lipid peroxidation (MDA content) is associated with cell membrane damage. Therefore, this result may indicate that the accumulation of ROS caused membrane damage.
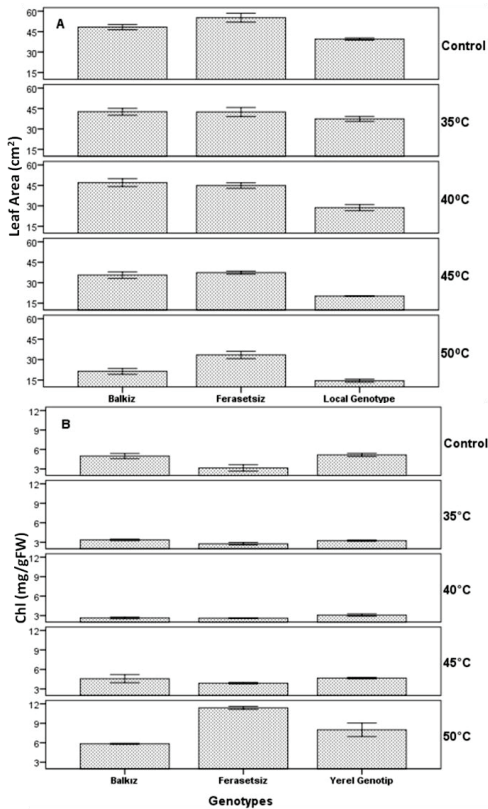
genotypes. There was a decrease in the TSP content of the 'Balkız' and 'Ferasetsiz' genotypes, at 50ºC. In the 'Local Genotype', the most significant reduction in TSP content was observed at 45ºC. A two-way ANOVA revealed significant effects of temperature, genotype and the interaction between temperature and genotype on TSP content (Table 1).
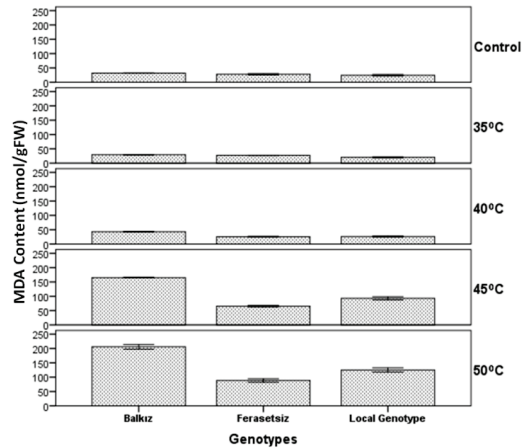

Figure 3. MDA content of three green bean genotypes in heat-stressed and control samples. Vertical bars indicate ±S.E. of three replications

Figure 2. Leaf area (Panel A) and Chl (Panel B) of three green bean genotypes in heat-stressed and control samples. Vertical bars indicate ±S.E. of three replications

In general, it was observed that the effect temperature applications on the total soluble protein (TSP) content of green bean genotypes in leaf tissues was different (Figure 4). Total soluble protein content of genotypes decreased significantly compared to control treatment at 40ºC. A clear increase at 45ºC was observed TSP content of the 'Balkız' and 'Ferasetsiz'
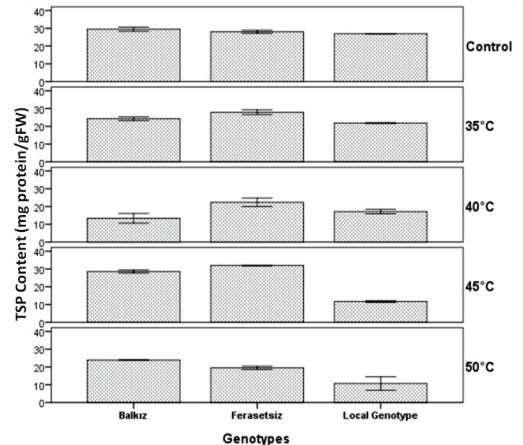

Figure 4. Total soluble protein content of three green bean genotypes in heat-stressed and control samples. Vertical bars indicate ±S.E. of three replications
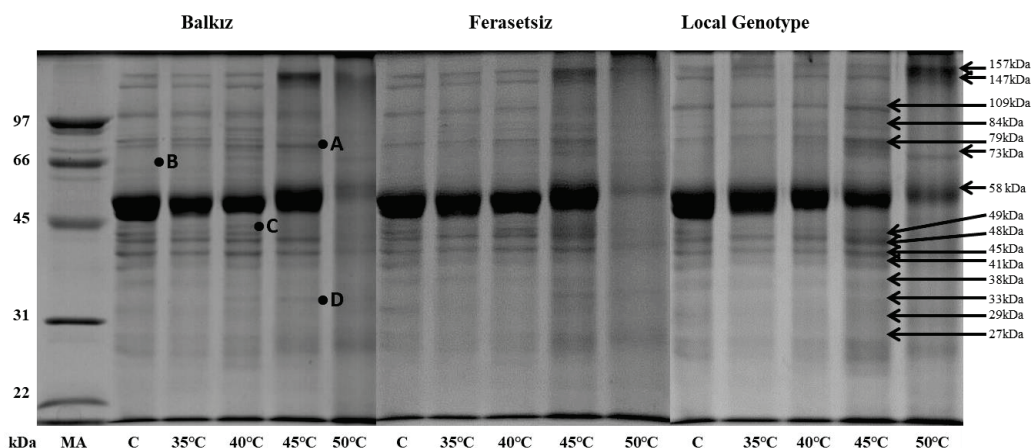
Figure 5. Effect of heat stress on protein profiles of green bean genotypes. MA: Marker, C. Control, A: 78 kDa; B: 65 kDa, C: 45 kDa, D: 34 kDa

Ledesma et al. (2004) reported that TSP content decreased, while new proteins had been synthesized depending on increased temperatures in "Nyoho" and "Toyonaka" strawberry varieties. High temperature stress also reduced the TSP content and increased the total amino acid and proline accumulation in mulberry (Chaitanya et al., 2001). Similarly, Turhan et al. (2015) determined that high temperature applications reduced the TSP content up to certain temperatures in pepper cultivars. High temperature stress leads to structural changes of proteins. Thus, proteins become denatured, proteolytic enzymes become sensitive (Levitt, 1980).

Figure 5 shows the total protein profiles in green been genotypes depending on high temperature applications. It has been determined that many protein bands, in mass range 27-157 kDa, were detected depending on the genotypes and heat treatments. Unlike the 'Ferasetsiz' and 'Local Genotype', in the 'Balkız' genotype, protein bands of 45, 65 and 78 kDa were determined and 34 kDA were different from the 'Local Genotype'. The density of the bands decreased in all three genotypes due to increasing temperature applications and this reduction was especially significant at 50°C. The 58 kDa protein band was the most frequently observed protein band in all three genotypes. It was determined that the 58 kDa protein band was probably a dominant band consisting of structural proteins

and this band decreased in all genotypes at 50°C. On the other hand, some protein bands were completely lost at 50°C. In addition, it was determined that the protein band of 45 kDa was synthesized at 40°C in 'Balkız' genotype and this band lost again at 45-50°C. This decrease in protein bands due to high temperature is thought to be due to the deterioration of protein structure at increasing temperatures. Similarly, it was reported that new proteins are synthesized or reduced and almost complete lost in tomatoes (Heckathorn, 1998) and strawberries (Ergin et al., 2016; Ledesma et al., 2004) in response to high temperature stress. On the other hand, Ergin et al. (2016) determined that two protein bands of 40 kDa and 23 kDa accumulated at high temperatures (from 46°C) in strawberry leaves in gradual and shock high temperature applications. Similarly, Turhan et al. (2015) found that strips of 7-54 kDa in the pepper plant under high temperature stress conditions and 40 kDa protein band may be associated with high temperature tolerance.

The calculated stress tolerance point (LT_{50}) values of three green bean genotypes are shown in Figure 6. Heat-acclimation caused an increase in heat-tolerance of all genotypes, which was the highest in 'Ferasetsiz' (LT_{50}; 50.8ºC) and 'Local Genotype' (LT_{50}; 49.9ºC). Meanwhile, 'Balkız' had the lowest heat-tolerance with a LT_{50} value of approximately 45.5ºC. A two-way ANOVA revealed

477

significant effects of temperature, genotype and the interaction between temperature and genotype on LT_{50} value (Table 1).
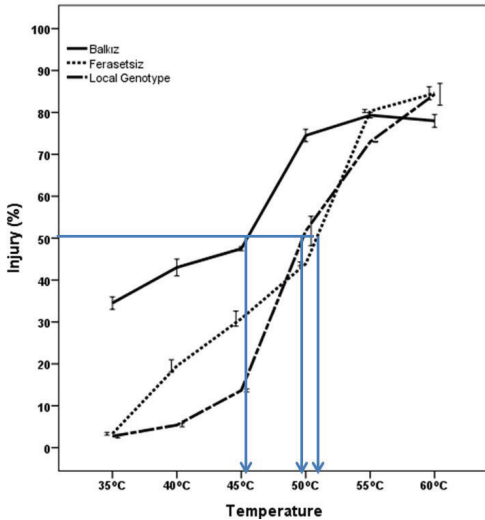

Figure 6. Heat stress tolerance (LT_{50}) of three green bean genotypes. Vertical bars indicate ±S.E. of three replications

In plants, measuring electrolyte leakage is a common evaluation way for cell membrane thermostability and membrane thermostability can be a valuable selection criterion for HST (Gulen & Eris, 2003). Electrolyte leakage is affected by the age, sampling part, development period, growing season, hardening and species of plant (Wahid et al., 2007). In this study, from the HST degrees of the genotypes, it can be suggested that 'Ferasetsiz' and 'Local Genotype' are relatively more tolerant to high temperatures than 'Balkız' genotype. Similar results have been found in strawberry cultivars by Kesici et al. (2013). Besides Cansev (2012) suggested that 'Gemlik' olive cultivar has moderate HST according to its LT50 value. Lethal temperature (LT_{50}) is the temperature at which 50% of individuals in a population are exposed to high damage or die. In a study, conducted on the *Deschampsia antarctica* Desv lawn variety it was showed that LT_{50} value as 48.3ºC due to membrane damage (Reyes et al., 2003). Turhan et al. (2015) demonstrated that, the LT_{50} values were determined as 36.9, 37.0 and 42.3 in 'Çarli', 'Çoban' and 'Demre' pepper varieties, respectively in the high temperature stress. They suggested that 'Demre' pepper cultivar

was more tolerant to heat stress according to its LT_{50} value.

CONCLUSIONS

The determination of the tolerance of genotypes for the breeding of high temperature or tolerant genotypes in beans is important in terms of obtaining efficient genotypes. This will be possible by explaining the mechanisms developed by the plants against heat stress. In summary, leaf RWC, loss of turgidity and MDA content were found to be effective in determining the tolerances of some green bean genotypes to high temperatures. The LT_{50} showed the heat-tolerance between 45.5 and 50.8ºC in the green bean genotypes. The genotypes 'Ferasetsiz' and 'Local Genotype' were determined as relatively heat-tolerant, while 'Balkız' was relatively heat-sensitive among the 3 green bean genotypes evaluated. The results obtained from this study could shed light on the molecular mechanism of heat stress and future studies on the development of heat-tolerant green bean genotypes.

ACKNOWLEDGEMENTS

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