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RESEARCH ARTICLE

Effects of low-temperature acclimation on morphological and physiological indices of banana seedlings

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ABSTRACT

Aim: Climate change has become the most important factor limiting the banana cultivation area, especially in countries with subtropical climates. To assess the effect of cold stress on banana, twenty-eight global and local cultivated clones were used for morphological and physio-biochemical evaluation. **Methods:** The banana clones were produced from shoot tips under *in vitro* conditions. When the plants were 20-25 cm in height and 5-6 leaves, they were transferred to climate-controlled rooms. The temperature of the testing room was gradually decreased every three days (from 28ºC day/22ºC night to 4ºC day/-1ºC night). After the seedlings remained at these temperatures, the temperature of the room was increased every three days (from 4ºC day/-1ºC night to 28ºC day/22ºC). When the treatment room reached control room conditions (28ºC day/22ºC night), measurements and analyses were started. **Results:** Low-temperature stress decreased pseudostem length, pseudostem diameter, leaf area, and leaf number of banana clones. The malondialdehyde contents (MDA) were increased compared with control; the chlorophyll content and fluorescence decreased significantly. When temperatures return to normal conditions (28ºC day/22ºC night), only eight banana clones managed to survive and twenty banana clones irreversibly died. After a gradual increase in temperatures, plants have continued to live and form new leaves. **Conclusion:** At the end of study, it was understood that the low temperatures applied would be sufficient to determine the low-temperature tolerance in banana clones and could work at lower temperatures.

Keywords: Chlorophyll; Electrolyte leakage; Lipid peroxidation; *Musa*; Tolerance; Visual scale

INTRODUCTION

Banana is an important crop in tropical and subtropical regions, believed to be originated from Indo-China and South-East Asia, where it has many wild species (*Musa acuminata* AA and *Musa balbisiana* BB) now (Simmonds, 1959). The temperature has a major impact on banana growth and development in Turkey. The most of banana growers produce 'Azman', 'Dwarf Cavendish', and 'Grand Nain' cultivars as cultivation material in Turkey and these cultivars have A genome (*M. acuminata*). 'Azman' cultivar is thought to be one of the clones of 'Grand Nain' mutated over time. However, unlike other cultivars, 'Azman' bends towards the outlet of the bunch and forms a white waxy powder layer on the pseudostem.

Low temperature (LT) tolerance varies between banana varieties and genotypes. Especially, genotypes with the A genome (*M. acuminata*) have a greater tolerance than genotypes with the B genome (*M. balbisiana*) (Israeli and Lahav, 2000). The effect of LT stress on the phenotype varies from plant to plant, but the most common symptoms are wilting, chlorosis, necrosis, restriction in leaf expansion (Lyons, 1973; Wilkonson et al., 2001; Mahajan and Tuteja, 2005). In optimum conditions, the banana plant can grow faster and comes to flowering even earlier than normal conditions. However, in winter (13-14ºC) the plant formation may a half leave per month and the leave formation may also stop when the temperature drops below 9-10ºC. Temperature is also a factor that affects the developmental view of banana plants. In warmer weather, the leaves develop horizontally, and in cold weather develop vertically (Ravi et al., 2013). In the open-field studies, plantains showed better cold tolerance than bananas. However, could not be obtained clear information about the physiological mechanism of plantains (Zhang et al., 2011) and Cavendish groups bananas (Sutherland, 2013). As it turns out, the response of banana plants to low temperature stress was tried to be determined morphologically and physiologically by many researchers. It also reported in some studies that application of important chemicals such as low concentration H_2O_2, Ca^{2+}, salicylic

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acid, brassinolide, or methyl jasmonate can increase cold tolerance and help avoid injury (Kang et al., 2002; Kang et al., 2003, Liu et al., 2008; Feng et al., 2009). However, in addition to the physiological assessment, recent studies have focused on determination of mRNA and protein level by proteomic (Yang et al., 2012; Feng et al., 2015) and transcriptomic (Yang et al., 2015; Liu et al., 2018) methods. Several cold stress related banana genes have been also identified from *in vitro* cultures subjected to low temperature stress (Santos et al., 2009) and a cold-resistance-related plantain gene (*MpRCI*) has been identified that enhances low temperature-resistance when heterologously expressed in transgenic tobacco (Feng et al., 2009).

Banana cultivation in Turkey is carried out especially in the Mediterranean Region. The temperatures used in the study were determined according to the highest, lowest and average temperature values of the last five years. In recent years, the average temperature of the region has decreased and the exit from the winter months has been prolonged. Despite the cultivation under the greenhouse, the producer is highly affected by frost damage every year. Because the development is delayed, the harvest continues until the winter months. Some plants come to a different environment from naturally growing areas, they can develop their cold tolerance by a mechanism called cold acclimation (Ruelland et al., 2009). Banana cultivation in Turkey began in the 1930s and economically important varieties throughout the years have been adapted to the climatic conditions. Banana cultivation in the coldest zone in the world is carried out in Turkey. In the present study, it was determined that the level of LT tolerance gained in Turkey climate conditions and the effects of LT on the leaves and pseudostem of seedlings at twenty-eight banana clones. It has also given theoretical information about the cold acclimation process and status of some variables involved in the physiological mechanism.

MATERIALS AND METHODS

This study was performed on 28 banana clones located in the Mersin city (34°E 36°N, sea level, average annual temperature: 23.3/14.7°C, mean relative humidity: 70%, mean annual precipitation:138 mm), Erdemli, in Southern Turkey (one of the most important areas of banana cultivation in the country). The clones used in the study were selected from different growing areas of Turkey. The average spacing between plants in the greenhouse was 3 m^2. The horticultural practices included irrigation and fertilization (45 kg of nitrogen, 150 kg of potassium, and 60 kg of phosphate per plant) for a year. 28 banana clones were exposed to a low-temperature acclimation experiment. However, only eight clones survived. Code numbers, the location from which they were selected, and the names of the clones are given in Table 1.

Multiplication of clones

The seedlings were obtained from shoot tips by micropropagation. Sterilization of explants and preparation of initial, multiplication, and rooting mediums were done according to Gubbuk and Pekmezci's (2004) protocol. Plants were watered with a standard nutrient solution (Turner, 1983) 3-4 times for a week during the experiment.

Low-temperature acclimation (LTa)

For LTa treatment, the temperature of climate-controlled room was gradually decreased every three days (28ºC day/22ºC night, 28ºC day/15ºC night; 22ºC day/11ºC night; 16ºC day/7ºC night; 10ºC day/3ºC night; 4ºC day/-1ºC night). After the samplings remained at these temperatures, the temperature of the room was increased every three days (4ºC day/-1ºC night, 10ºC day/3ºC night, 16ºC day/7ºC night, 22ºC day/11ºC night, 28ºC day/15ºC night, 28ºC day/22ºC night). When the room temperature reached to control room conditions (28ºC day/22ºC night), measurements and observations were started. The control plants were placed in another climate-controlled room and the temperature was 28ºC day/15ºC night and 2000 lx light intensity. The relative humidity (RH) of the two rooms was kept at 80% during the process.

Assessment with 0-9 scale

No visual scale has been detected in the banana LT stress studies so far. For this study a visual scale was created to reveal the degree of morphological damage in seedlings;

0: No effect on plants (control plants), 1: A slight wilt in plants (According to control plants), 2: Partial (half of the leave) beginning of wilting on the first leave (bottom leave), 3: Partially wilting on the first and second leaves, 4: Wilt and redness from first leave to fourth leave, 5: Fading, redness and a noticeable slowdown in growth from first leave to fourth leave, 6: Partially wilt and beginning of drying leave edges from first leave to fifth leave, 7: Severe paleness, and redness from first leave to fifth leave, 8: Wilt, redness, and drying of all leaves of the plant, 9: Severe burns and

Table 1: The list of origins and code numbers of living clones

Code Number	Origin	Cultivar Name	Scientific Name
BZ 004	Bozyazı	Azman	*Musa acuminata*
G004	Alanya	Azman	*Musa acuminata*
ETK11	Anamur	Azman	*Musa acuminata*
AN205	Alanya	Azman	*Musa acuminata*
AN 006	Anamur	Dwarf Cavendish	*Musa acuminata*
AN001	Gazipasa	Azman	*Musa acuminata*
AL15	Anamur	Dwarf Cavendish	*Musa acuminata*
AL004	Anamur	Azman	*Musa acuminata*

death of plants. The classification was made according to the following visual scale: 0-3: tolerant, 4-6: medium level tolerant, 6-8: sensitive, 9>: highly sensitive.

The pseudostem length and diameter

The area was measured from the root throat to the shoot tip by meters as cm (± 0.5). Pseudostem diameter was determined as mm (± 0.1) with the help of a digital caliper.

The leaf number and area: The number of leaves was counted as the number of leaf/plant; leaf area was determined as cm^2/plant by using the Licor LI-3000A model leaf area meter. The remaining green parts of the leaves were carefully cut with scissors. Newly formed leaves were not considered.

Chlorophyll fluorescence

SPAD values were recorded using a SPAD-502 meter (Konica-Minolta, Japan).

Net Photosynthetic capacity

Photon-saturated gas exchange was measured on leaf with a portable infrared gas analyzer (LI-6400, LI-COR, Lincoln, NE, USA). During measurements, the relative humidity was about 80% and leaf temperature was maintained at room temperature for each plant.

Total Chlorophyll Content

Total chlorophyll (TC) analysis was performed according to the protocol of Arnon (1949). The TC content in the samples was measured at $A_{652\,nm}$ by spectrophotometer and calculated as mg per fresh weight (g).

Lipid peroxidation

The malondialdehyde (MDA) content was estimated according to Sun et al. (2006). The supernatant was analyzed with a spectrophotometer and calculated from A_{535}-A_{600} using the coefficient of absorbance of 155 $mM^{-1}cm^{-1}$ (Lei et al., 2010).

Membrane Injury Index

The percentage of electrolyte leakage was measured to evaluate the degree of LT injury in the leaf of banana seedlings. Five leaf discs (diameter: 1.3 cm) were collected from treatment and control plants. They were immersed in deionized water for 1.5 h at room temperature, followed by a 30 min boiling treatment. The conductivity of leaked electrolytes before and after boiling was determined by using a 4520 Conductivity Meter (JENWAY, Dunmow, Essex, UK). Electrolyte conductivity (C2) was measured, and the relative electrical conductivity (C%) was calculated with the formula; (C1/C2) × 100.

Statistical analysis

Data were presented as mean±SDA and subjected to two-way ANOVA with randomized plot design with fifteen replications for each parameter using JPM 5.0.1. software (SAS Institute, Cary, NC, 1989) followed by the LSD test (P<0.05).

RESULTS

The effects of LTa were investigated in twenty-eight banana clones. Eight of the seedlings periodically exposed to different temperatures survived when temperatures return to normal conditions (28ºC day/22ºC night), while the other twenty banana clones have irreversibly died. Six of the surviving clones were 'Azman' and two were 'Dwarf Cavendish' cultivars. A visual scale (0-9) was used to determine the level of morphological damage on the clones. According to the scale, 'BZ004' had a minimum injury, whereas maximum injury was recorded at 'G004' (Fig. 1). The mean values of the scale varied between 3.75 and 8.00 (Table 2).

The pseudostem diameter (PD) of all clones was reduced by the LTa treatment, significantly for 'G004' and 'AN006'. The largest PD values were obtained from 'AL004' (25.54 mm) and 'ETK11' (25.33 mm) within LTa treated group and 'BZ004' (41.10 mm) and 'G004' (40.00 mm) within the control group. Similarly, pseudostem length (PL) also decreased after LTa. The highest PL was observed in 'AL004' and 'ETK11' within LTa treated group (Table 3). Similar to the PD results, the same clones had the highest PL in the treated group. However, the development level of the control group clones was changed.

Leaf number (LN) was reduced by the LTa treatment. The most LN were observed in 'BZ004' (2.50) and 'AL004' (2.50) in the treated group (Table 3). The vast majority of treated plants lost almost more than half of their leaves after stress temperatures. LT sharply reduced leaf area formation. In the leaf area (LA) measurement made on seedlings, 'BZ004' had the least cold injury whereas 'G004' suffered the most severe injury (Table 3). All surviving clones showed a more than 90% decrease in leaf area compared to control plants.

'BZ004' and 'AL004' from the treated group showed the highest total chlorophyll (TC). It was also examined the chlorophyll fluorescence (CF) in plants. The CF and TC were found to be very less in treatment plants compared to the control group (Table 3). The highest values for CF were detected in 'BZ004' in both groups.

After LTa treatment, the values of the net photosynthetic capacities (NP) in the seedlings decreased according to the control plants (Table 3). The NP values were found significantly different in eight banana clones. Especially,

Fig 1. Morphological damage of clones after stress temperatures (Images represent replicates and visual scale; (a) BZ004, (b) AL004, (c) ETK11, (d) AN205, (e) AL15, f. AN001, (g) AN006, (h) G004

'BZ004' from treated group plants had the highest NP value.

'AL15' and 'G004' from the treated group plants and 'G004' from the control group plants had the most content of malondialdehyde (MDA), while 'AL004' and 'BZ004' from the treated group and 'AN006' from the control group had the least content of MDA (Table 3).

The relative leakage changed from %3.36 to %45.33 in the banana leaves. The most and least *C*% were detected in 'G004' and 'BZ004' in the treated group, respectively. These observations suggest that the seedlings of 'BZ004' had adapted to low-temperature stress temperatures than the other clones (Fig. 2).

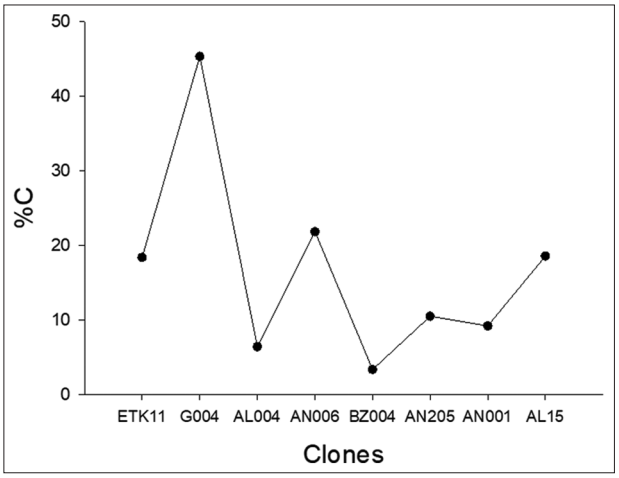

Fig 2. Effects of low temperature treatment according to the control plants on the membrane damage index

DISCUSSION

There are very limited data in the literature on the low-temperature tolerance of members of the *Musaceae* family to which banana belongs due to the favorable climatic conditions of the predominantly grown countries. The most of banana production regions are in the southern subtropical which is at the northern zone of world banana cultivation. The banana production often suffers chilling and freezing damages in winter and also in spring when extreme weather conditions occur. The materials produced by tissue culture had 77% more root system and 99% more leaf area than the materials produced by conventional manner. This was an indication of better nutrient delivery from roots to shoots (Eckstein and Robinson, 2015). *In vitro* culture materials were used in the study and it can be said that the studied plants may have better tolerance to LT stress than conventional materials in terms of nutrient transportation.

Table 2: Scale averages in banana clones (Scaling values are increasing from 0 to 9 and damage to plants is increasing)

Clones	Scale score	Tolerance Level
AL004	5.50	medium level tolerant
AL15	7.50	sensitive
AN001	5.50	medium level tolerant
AN006	6.75	sensitive
AN205	5.50	medium level tolerant
BZ004	3.75	tolerant
ETK11	6.75	sensitive
G004	8.00	sensitive

Researchers have been trying to discover cold-resistant genes and developing cold-resistant banana cultivars and

Table 3: Effects of low temperatures on the psedudostem diameter (PD), pseudostem lenght (PL), leave number (LN), leave area (LA), chlorophyll fluorescence (CF), net photosynthetic rate (NP), malondialdehyde (MDA) and total chlorophyll (TC) in the eight banana clones.

Treatment	Clones	Parameters							
		PD (mm)	PL (cm)	LN (n)	LA (cm^2)	CF (Fv/F)	NP ($\mu mol\ (CO_2)\ m^{-2}s^{-1}$)	MDA ($\mu mol\ g^{-1}$)	TC ($mg\ fw^{-1}$)
Control	AL004	38.41±0.63^{bc}	39.54±1.38^{b}	6.50±0.58	841.80±5.34^{a}	0.47±0.00^{a}	2.21±0.01^{cd}	5.22±0.00^{k}	0.347±0.005^{e}
	AL15	37.00±1.73^{cd}	38.31±1.16^{c}	5.75±0.50	724.73±7.57^{d}	0.40±0.04^{cd}	2.79±0.05^{b}	5.21±0.82^{n}	0.572±0.004^{bc}
	AN001	34.72±2.12^{e}	37.86±0.51^{c}	5.25±0.50	833.12±8.50^{b}	0.42±0.03^{bcd}	2.71±0.14^{b}	5.22±0.96^{l}	0.582±0.003^{b}
	AN006	35.72±2.21^{de}	38.39±0.35^{c}	5.25±0.50	764.94±2.44^{d}	0.42±0.04^{bcd}	2.71±0.07^{a}	4.46±1.73^{p}	0.537±0.030^{c}
	AN205	38.56±0.19^{bc}	38.83±0.18^{bc}	6.00±0.00	845.43±3.85^{a}	0.40±0.05^{bcd}	3.29±0.07^{a}	5.22±0.96^{m}	0.564±0.010^{bc}
	BZ004	41.10±0.96^{a}	43.73±0.45^{a}	7.50±0.58	830.61±9.78^{c}	0.43±0.04^{bc}	3.29±0.13^{a}	5.16±1.50^{o}	0.693±0.020^{a}
	ETK11	38.34±0.90^{bc}	23.87±0.79^{e}	6.50±0.58	729.11±8.96^{d}	0.40±0.00^{d}	2.29±0.27^{c}	5.26±0.96^{j}	0.401±0.013^{d}
	G004	40.00±1.02^{a}	38.78±0.17^{bc}	6.00±0.00	649.93±6.55^{e}	0.43±0.01^{b}	2.65±0.01^{b}	5.77±0.58^{i}	0.332±0.001^{e}
Low Temperature	AL004	25.54±1.84^{f}	23.24±1.31^{e}	2.50±0.58	55.06±2.80^{hi}	0.23±0.002^{fg}	1.82±0.01^{ef}	6.15±1.41^{g}	0.266±0.002^{f}
	AL15	23.28±1.47^{gh}	20.32±0.37^{g}	1.50±0.58	43.80±3.27^{jk}	0.26±0.01^{ef}	1.78±0.01^{efg}	8.83±0.82^{a}	0.115±0.020^{ij}
	AN001	23.36±1.38^{gh}	21.67±1.17^{f}	1.50±0.58	54.16±2.99^{hi}	0.23±0.04^{g}	1.75±0.04^{efg}	7.18±0.50^{e}	0.184±0.0013^{gh}
	AN006	22.03±0.64^{h}	19.58±0.29^{g}	1.00±0.00	47.64±3.30^{ij}	0.18±0.01^{h}	1.77±0.01^{efg}	7.66±0.58^{c}	0.153±0.001^{hi}
	AN205	24.24±2.77^{fg}	22.11±1.24^{f}	1.75±0.96	67.87±1.38^{g}	0.23±0.01^{g}	1.76±0.04^{efg}	6.77±1.50^{f}	0.220±0.014^{g}
	BZ004	24.35±0.90^{fg}	25.51±0.37^{d}	2.50±0.58	77.75±5.57^{f}	0.28±0.01^{e}	1.99±0.00^{de}	5.20±0.50^{h}	0.266±0.003^{f}
	ETK11	25.33±0.56^{f}	23.87±0.67^{e}	2.25±0.50	56.90±1.66^{h}	0.26±0.01^{ef}	1.69±0.56^{fg}	7.46±0.50^{d}	0.173±0.100^{h}
	G004	22.03±0.26^{h}	20.11±0.59^{g}	1.00±0.00	38.63±0.64^{k}	0.18±0.01^{h}	1.55±0.03^{g}	8.81±0.58^{b}	0.104±0.01^{j}

Data are the mean ± SDA. Values represent the means of fifteen independent biological replicates. Significant differences between means are shown by different letters ($P \leq 0.05$)

sometimes by protecting the banana from chilling or freezing using cold-resistance measures (Yang et al., 2015). However, the powerful studies and measures so far have not been enough (Liu et al., 2015). In the study, there was a significant decline in plant height, plant diameter, and leave development after stress temperatures. Yang et al. (2015) reported that after 6 h and 48 h cold treatment at 10°C, the banana leaves drooped. The second and third leaves from down to up showed heavy necrosis and wilting symptoms. In the study, although the plants were accustomed to different low temperatures, similar effects were observed and a significant difference was also observed between the degrees of damage.

In the initial stages of plant development, after the abolition of low-temperature stress, the plants can be recovered and returned (Ashraf and Harris, 2004). A gradual acclimation to stress temperatures can induce physiological adjustments in the plants that protect from them injury after unexpected environmental stresses (Weiser, 1970). It has been suggested that to obtain proper freezing tolerance and reach maximum tolerance, temperatures may need to be changed gradually. The height, tendency, and coloration of the pseudostem depend on the cultivar and the cultivation conditions (Pillay and Tripathi, 2007). Similar to these findings, the morphology of some clones was severely affected after LTa temperatures, but others were less affected. Studies have shown that cold stress directly affects root activity (Choi et al., 1995). The present study shown that the clones that died irreversibly were easily removed from the soil when they have held the pseudostem. On the contrary, the others were still holding on to the soil firmly.

The leave numbers of clones have changed from 1.00 and 7.50. The most important factor affecting banana yield is the number of leaves. Robinson (1996) reported that when the temperature increase from 17°C to 19°C, the leaf number per plant increases. Sub-zero temperatures cause the water in plant tissues to turn into ice. Ice crystals formed in extracellular spaces reduce the water potential of the apoplastic solution which leads to water flowing from the cells (Ritonga and Chen, 2020). With the stress caused by ice, water stress occurs in the plant. In the study, it can be thought that this is one of the main reasons for the drying of plant leaves. However, after LTa temperatures were disappeared, the seedlings continued to form new and fresh leaves. Additionally, the observations showed that the older leaves (with or without coloration) of LT-acclimated plants tend to withstand a few days after the plants are moved back to normal growth temperatures, although newly emerged leaves (with or without coloration) continued to grow. Consistent with the results of this study, Patel et al. (2016) reported that multistep conditioning was usually more effective in the banana plant. Gradually decreasing the temperature in 3°C steps from 21 to 5°C at 12 h intervals resulted in the least chilling injury compared to 5°C decreases every 24 h, an 8°C decrease every 36 h or a single changes from 21°C to 5°C. The temperatures selected in the study were calculated according to the average temperatures of the last five years

and the average monthly temperature of these years. The duration of the temperatures was also calculated in this way. The critical temperature created to determine the tolerance of the clones and to separate them from each other was 4°C day/-1°C night for 3 days. Therefore, after stress temperatures, 20 out of 28 clones were eliminated. Functional genes known as heat shock proteins expressed under cold stress repair plant cells by the denaturation of proteins (Vinocur and Altman, 2005). The reason why eight of the clones used in the study survived may be related to the number and expression level of these functional genes. However, it needs to be investigated in more detail.

The level of damage occurring in the leaves will vary according to the low temperature and period. The optimum degree for banana plants is 27°C and it increased the rate of leaf formation and net assimilation (Robinson, 1996). However, leaf production was decreased 51% compared to control plants after LT stress and delayed leaf development for 11 days (Damasco et al., 1997). In the study, considering the duration of the LT, the control group plants increased the LA by over 90% compared to the treatment group plants.

The leaf chlorophyll content is an important factor that is often measured as an indicator of chloroplast development, photosynthetic capacity, leaf nitrogen content (Ling et al., 2011). In our study, with prolonged low-temperature stress, TC contents of both clones continued to decrease and were lower than those of the plants maintained under 28ºC day/22ºC night conditions, as reported previously. El-Mahdy et al. (2018) reported that a significant deterioration of total chlorophyll was clear after 48 h cold stress at 5°C which was by 48 and 20.6% compared to control, for Grand Nain and Williams cultivars, respectively. In the study, the average TC ratio of treatment groups decreased by almost 63.29% compared to the control. The leaf profile (young-aged), leaf surface, plant growth (pre-flowering, fruit formation, harvest), varieties, type of grown material (sucker, in vitro propagated plant) can affect the photosynthesis rate. To avoid variable factors, the NP measurements were conducted on the green part of third leaves in the study. Zhang et al. (2011) reported that after treatment for 48 h, the values of PN in the saplings of two cultivars decreased by 54.0% and 53.9%. In our study, the rate of PN decreased by 35.77%. This may be due to the gradual acclimatization of plants to normal temperature conditions. Liu et al. (2018) preferred temperatures close to stress temperatures (28°C, 20°C, 13°C, 4°C, 0°C, -2°C, -4°C, and -6°C) of our study. After temperature treatment for 24 h, the field and in vitro cultured plants were used to understand the morphological changes of the leaves of 'Sanming' wild banana in all the temperature conditions tested. However, the *in vitro* plantlets and leaves showed severe damages like serious water-logging, wilting, and death under the treatment of -2°C and down -2°C. Although the clones used in the study showed similar symptoms, we had healthier plants because of gradually decreased temperatures. In addition, the fact that the plants were more mature may have had a positive effect.

Malondialdehyde (MDA) is also considered a reliable sign of plant oxidative stress and the organic unity of the membranes in response to low temperatures. In the study, MDA content in leaves of clones increased with the low temperatures, especially in the LT-sensitive AL15, AN006, ETK11 and G004 clones which showed more MDA accumulation in the cell membranes of the leaves. MDA analysis showed that the average content of the seedlings was changed between 5.10 μmol/g (control) and 7.25 μmol/g (treatment). He et al. (2018) reported that MDA contents varied 15-25 nmol/g at 10°C for 0, 3, and 6 h. While these values were significantly lower than our findings, the plants had similar characteristics in terms of carrying MDA before they exposed the stress temperatures. The MDA values were more than the findings of Zhang et al. (2011), who found a considerable reduction in the photosynthetic performance by 54.0% and 53.9% in two cultivars after exposed at 7°C for 48 h. According to results, the MDA content of the treatment group decreased by almost 29% compared to the control group. In addition, MDA content was found to be high in control group plants. The high MDA content in control plants may have emerged from a process of acclimation to Turkey. Generally, plants from temperate regions have a cold acclimation tendency, and they can develop increased freezing tolerance after being exposed to low, nonfreezing temperatures (Nakashima and Yamaguchi-Shinozaki, 2005). Sreedharan et al. (2013) reported that the MDA levels of untransformed banana leaves were the highest, indicating that the transgenic plants differ not only from the untransformed controls in their recovery capacity but also in the rate of damage exposed progressively during the cold stress period. Dou et al. (2016) reported that the MDA accumulation was considerably less in the transgenic banana lines than in the wild type at LT conditions. Before cold treatment, plants had similar MDA levels. When the results of these studies were evaluated, it was found that the pre-stress plants had a certain MDA content, which was compatible with this study. When the mechanism of low temperature stress of the clones used in the study is evaluated, they continue to live when the low temperature factor is removed. It was observed that the MDA content tended to return to the amount in the control conditions. It is also thought to be important membrane fluidity in stress tolerance.

High relative humidity (RH) condition (90%) is more important for banana cultivation and allow the chilling

injury to be overcome slightly and delayed the increase of membrane leakage rate and MDA content on banana seedlings than at the middle RH condition (60%-70%) (Zhang et al., 2011). This information has been taken into account and kept RH at 80% during the study. Cold stress damages the cell membranes (Rohde et al., 2004), and cell membrane permeability is evaluated according to the cell membrane conductivity of stressed plants (Steponkus et al., 1990). The electrical conductivity test is based on increasing the electrolytic conductivity of ions (especially K+) from the cell as a result of damaging the cell membranes and is performed to compare the membrane integrity between plants (Murray et al., 1989). LT damage estimated by electrolyte leakage, has been widely used as a parameter identifying the cold tolerance of plant tissues. When the ice crystals formed in the plant tissues after cold stress, lead to an increase in electrolyte leakage and membrane lipid phase changes. To ensure freeze tolerance of plants during the freeze-thaw cycle, it is necessary to increase cell membrane durability (Uemura et al., 2006). In the study, an electrical conductivity test was made and *C*% was calculated according to the results. The relative leakage changed from 3.36% to 45.33% in the banana leaves. Accordingly, it can be said that the cellular dehydration of the G004, ETK, and AN006 varieties is the highest after stress. Yang et al. (2015) reported that the relative leakage increased from 12.0 to 33.9% in banana leaves, and from 14.6 to 28.0% in plantain leaves after 6 h of cold treatment at 10°C. Dou et al. (2016) reported that at the end of 10°C 48-hour cold treatment, the electrolyte leakage of wild type plants was 77.6%, which was significantly higher than that of the Dajiao and transgenic lines: 51.6% for Dajiao, 59.1% for #2 and 55.8% for #5, indicating that cell damage was more serious in the wild type. Although C% values were evaluated at lower temperatures in this study, they were close to the results of other studies.

When the variables used in the study were evaluated among themselves, the MDA content and membrane injury index content increased while plant height, plant diameter, leaf number, leaf area, chlorophyll fluorescence, total chlorophyll and net photosynthesis rate decreased. When evaluated on a clone basis, less MDA content and membrane damage index were determined in tolerant and moderately tolerant clones. Cold adaptive plants always store more sugar in their underground tissues (Londo et al., 2018; Janska et al., 2010) and the most popular approach used by plants to deal with LT stress is cold acclimation, which allows plants to survive freezing via accumulation of cryoprotective polypeptides and osmolytes (Khan et al., 2015). More detailed screening of these clones in terms of the aforementioned compounds in the future studies will be beneficial in terms of elucidating cold stress mechanism.

CONCLUSION

This study was designed to illuminate the low-temperature tolerance of banana clones growing in Turkey. 4°C day/-1°C night temperatures showed a destructive effect on twenty-eight banana clones and eight of them were lived. Especially, 'BZ004' gave better values than other clones in terms of some parameters. That is to say, in a long time of evolution, it should have some cold tolerance mechanism. It was also determined that these temperatures could be carried out by pulling down the temperature as 4°C day/-1°C night temperatures were sufficient to determine low-temperature tolerance in bananas.

Low temperature stress can actually be described as the drying of the plant. When the results discussed so far are evaluated, it can say that the plants used in the study increase cell membrane durability over time. When the temperature drops suddenly, applications to increase membrane durability can be beneficial. However, the duration of the low temperature the plants will also affect this process.

AUTHOR CONTRIBUTIONS

Baysal F. designed of the study and wrote all sections of the manuscript.

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