EFFECTS OF LOW TEMPERATURES ON ENZYME ACTIVITY, CHLOROPHYLL AND ION CONTENTS IN COMMON BEAN GENOTYPES

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Abstract. Low temperatures slow down plant growth and development and generate physiological damages on plants. Metabolism of largely produced and consumed common bean (*Phaseolus vulgaris* L.) plants decelerates at temperatures below 15 °C. This study was carried out at Van Yuzuncu Yil University in Van province of Turkey with limited vegetation period due to its cold climate in winter. In this study, the bean plants were subjected to low temperature stress and catalase (CAT), super oxide dismutase (SOD), ascorbate peroxidase (APX) enzyme activities, malondialdehyde (MDA), chlorophyll-a, chlorophyll-b and total chlorophyll, K, Ca, and Mg ion contents of low temperature stress-tolerant Yakutiye and Ç30 and low temperature stress-sensitive Zulbiye and Ç13 bean genotypes were measured on 25th, 30th, 35th and 40th days of low temperature stress. Four bean seeds were sown in 2-liter pots filled with 2:1 peat:perlite mixture as to have 2 plants in each pot. The pots were irrigated with Hoagland nutrient solution. Low temperature stress plants were sown on 15th of March with low outdoor temperatures. In control group, been seeds were sown on 25th of April and plant samples were taken in again 5-day intervals in 4 periods (25th, 30th, 35th and 40th days of growth). Significant differences were observed in enzyme activities, chlorophyll contents and ion contents of low temperature-tolerant and sensitive bean genotypes in 4 measurement periods.
Keywords: *abiotic stress, biochemical characteristics, nutrient, Phaseolus vulgaris, physiological characteristics*

Introduction

In terrestrial climate zones, low bean yields are generally attributed to prominent climate conditions of the regions. Seed germination in beans largely depends on genotypes and quite a few genotypes are able to well-germinate at temperatures below 10 °C. In vegetables, low temperatures at root zone generate a stress in water absorption of the roots (Sakamoto and Suzuki, 2015). Beans are generally sensitive to cold temperatures, thus germination rates and percentages decrease at low soil temperatures in spring. Water absorption damage increases because of rapid water intake and ultimately seedling formation greatly weakened. In cold climates, temperatures often go below 15 °C during the initial growth stages of the seedlings and such low temperatures then negatively influence seedling growth and development (Elkoca et al., 2005). Plants develop different mechanism against cold stress-induced damages and ultimate die outs or to provide cold-tolerance. Exothermic effects of inner and intra cell frost formation on plants are categorized as physical changes in cell membranes throughout cold-stress period, cold resistance and acclimatization and biochemical changes throughout the cold acclimatization (changes in some soluble substances in plant extract, lipid composition of cell membranes, protein contents, enzyme activity and antioxidant systems (Aslantaş et al., 2010).

Abiotic stress factors negatively affect several crops. Significant decreases were reported in photosynthesis rates and chlorophyll contents of pea plants when the plants were subjected to 45 °C temperature for 24 h (Georgieva et al., 2007). Decreasing

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chlorophyll, K and Ca contents and increasing MDA, CAT, SOD and APX activities were reported for bean and tomato plants under drought and high temperatures (Terzi et al., 2010; Kabay and Şensoy, 2016, 2017; Alp and Kabay, 2017).

It was reported in a previous study investigating the effects of low temperature stress on physiological characteristics of tomato genotypes that cold stress reduced chlorophyll and dry matter content of young and old leaves and wakened antioxidative systems of the plants (Gökmen, 2006). Low temperatures were reported to increase antioxidant enzyme and MDA content of rapeseed plants (Xian et al., 2017). Low temperatures increase chlorophyll and weight losses of cabbages and increased reactive oxygen species (Soengas et al., 2018).

It was indicated in another study carried out with cold stress-resistant and sensitive sugar cane cultivars that sensitive cultivar had greater root length and root volume than the resistant cultivar, but cell structure was destructed in both cultivars and cold stress-induced plants had greater MDA, proline, soluble sugar, soluble protein, POD and SOD activity than the control plants (Sun et al., 2017).

It was reported in another study investigating the effects of cold stress on rye and wheat seedlings that cold stress increased antioxidant enzyme activities, proline, sugar and anthocyanins contents of both species (Kolupaev et al., 2016).

Significant decreases were reported in malondialdehyde, proline, peroxidase and catalase activities of control and transgenic tomato plants under cold and low temperature stress (Yu et al., 2015). Xintaimici and Jinyan cucumber (*Cucumis sativus* L.) cultivars were subjected to two different temperature regimes (15/15 °C and 25/18 °C) under low light intensity and significant increases were reported in leaf superoxide dismutase and guaiacol peroxidase activities of both cultivars and less increases were reported in leaf catalase activity (Xu et al., 2008). Ogweno et al. (2009) reported different photosynthetic physiological responds of tomato leaves to moderate and low temperature stress. Huang et al. (2018) reported decreasing net photosynthetic rate and chlorophyll contents of Akihime and Benihoppe strawberry cultivars under cooling stress. Researchers also reported that foliar selenium (Se) sprays relieved the decrease in net photosynthetic rate and chlorophyll content and increased malondialdehyde and hydrogen peroxide content under stress conditions. Do et al. (2018) reported increasing ascorbate peroxidase (APX) and superoxide dismutase (SOD) activities of melon and pumpkin plants when they were subjected to 12/7 °C (day/night) low temperatures for 0–72 h. Li et al. (2018) reported that cold stress increased the accumulation of reactive oxygen species (ROS) and resulted in an oxidative stress, such a case then decreased photosynthetic processes of tea leaves, but melatonin treatments reduced antioxidant potential and oxidative stress and increased photosynthetic capacity. Bilska-kos et al. (2017) reported that low temperatures (14/12 °C) altered pectin content and osmatic potential of maize leaf cells and ultimately altered cell membrane composition. Zhou et al. (2017) reported significant decreases in fresh and dry weights, leaf areas and relative water contents of tomato cultivars under drought stress as compared to the control plants. While significant increases were reported in antioxidant compound quantities of tomato plants, decreases were reported in chlorophyll contents (Gökmen, 2006). It was reported in study carried out to determine yield and total temperature demands of bean plants at different growth stages that the greatest yield was obtained from early July sowings and yields decreased as the sowing time approached to autumn (Yoldaş and Eşiyok, 2009). High and low temperatures (15/8, 20/13, 28/21, 33/23 and 36/26 °C) were applied to tomato plants

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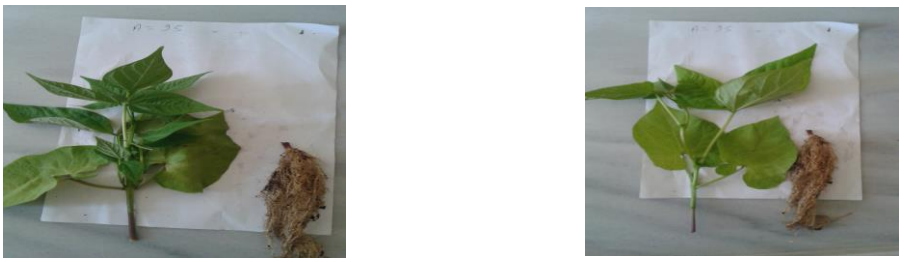
and it was observed that temperature stress negatively influenced plant growth and development and nutrient uptake (Sung et al., 2015).

This study was carried out to determine the effects of low temperature stress on enzyme activity, chlorophyll, K, Ca and Mg contents of low temperature tolerant (Yakutiye and Ç30) and sensitive (Zulbiye and Ç13) common bean (*Phaseolus vulgaris* L.) genotypes.

Materials and methods

This study was conducted to determine the effects of low temperature stress on common bean genotypes. The study carried out at the Greenhouses of Van Yuzuncu Yil University Erciş Vocational Collage of Turkey and the plant analyses were carried out at laboratories of the Agricultural Faculty of the same university. Catalase (CAT), super oxide dismutase (SOD), ascorbate peroxidase (APX) enzyme activities, malondialdehyde (MDA), chlorophyll-a, chlorophyll-b and total chlorophyll, K, Ca, and Mg ion contents of low temperature stress-resistant Yakutiye and Ç30 and low temperature stress-sensitive Zulbiye and Ç13 bean genotypes (*Fig. 1*) were measured on 25th, 30th, 35th and 40th days of low temperature stress. For low temperature stress, bean seeds were sown in 2-liter pots filled with 2:1 peat:perlite mixture on 15th of March with low outdoor temperatures in a plastic covered high tunnels. Four seeds were placed in each pot as to have 2 plants in each pot. Pots were irrigated with Hoagland nutrient solution. The initial samples were taken from 25-days old plants and high tunnel cover was removed. Plants were then exposed to outdoor conditions and plant samples were taken in 5-day intervals (25th, 30th, 35th and 40th days) in 4 periods. High-tunnel temperature was 16.76 °C in day time and 15.28 °C at night. When the tunnel cover was removed, day temperature was measured as 12.67 °C and night temperature was measured as 8.43 °C by Hobo temperature data loggers. In control group, bean seeds were sown on 25th of April into the pots placed in a high-tunnel and plant samples were taken in again 5-day intervals on 25th, 30th, 35th and 40th days of growth. Throughout the experiments, day temperature was measured as 27.44 °C and night temperature was measured as 16.87 °C. The analyses performed on plant samples are provided below.



Control Low temperature

Figure 1. *A view of control and low temperature stressed plant sample*

Mineral element analyses

At the end of 25th, 30th, 35th and 40th days after sowing, representative control and low temperature stress-treated plants were harvested, the shoot were initially dried at open places for two days and then dried in an oven at 65 °C for 48 h. About 200 mg of

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dry shoot samples were ground and subjected to pre-digestion in ethyl alcohol and then burned to ash in an ash oven at 550 °C. Resultant ash was dissolved in 3.3% HCl, filtered through blue-band filter paper. K, Ca and Mg readings were performed in an atomic absorption device at Scientific Research and Implementation Center of Yüzüncü Yıl University (Kacar, 1994; Kacar and İnal, 2008).

Lipid peroxidation

Lipid peroxidation of the plants generally expressed as malondialdehyde (MDA) content. About 0.5 g leaf sample was homogenized in 10 ml 0.1% trichloric acid (TCA) and resultant homogenate was centrifuged at 15000 rpm for 5 min. About 1 ml was taken from the clear portion of the centrifuged sample and supplemented with 0,5% tiobarbituric acid (TBA) dissolved in 4 ml 20% TCA. The mixture was kept at 95 °C for 30 min and instantly cooled in ice-bath. Cooled sample was then centrifuged at 10000 rpm for 10 min and absorbance readings were performed in clear portion at 532 and 600 nm wave lengths. The following equation was used to calculate malondialdehyde (MDA) content of the samples (Heath and Packer, 1968; Sairam and Saxena, 2000):

$$\text{MDA (nmol ml-1)} = [(A532\text{-}A600)/155\,000]\ 10^6$$

Antioxidative enzyme analyses

About 1 g frozen leaf sample was homogenized in 5 ml cold 0.1 M Na-phosphate, 0.5 mM Na-EDTA and 1 mM ascorbic acid mixture (pH: 7.5) and the resultant homogenate was centrifuged at 18000 rpm and 4 °C for 30 min. Ascorbate peroxidase (AP) activity of the resultant homogenate was instantly determined. For catalase (CAT) and superoxide dismutase (SOD) activities, 1 g frozen leaf sample was homogenized in 5 ml cold 0.1 M Na-phosphate and 0.5 mM Na-EDTA mixture (pH: 7.5). Resultant homogenate was centrifuged at 18000 rpm and 4 °C for 30 min. A portion of homogenate was used to determine CAT activity instantly and the remaining portion was kept at -20 °C for SOD activity (Jebara et al., 2005; Bagcı, 2010).

Catalase (CAT) activity

Catalase activity was determined through monitoring the inhibition of H_2O_2 at 240 nm. As the reaction solution, 0.05 M phosphate buffer (KH_2PO_4) and 1.5 mM H_2O_2 mixture was used (pH: 7.0). About 2.5 ml reaction solution was mixed with 0.2 ml plant extract. Readings were performed in a spectrophotometer at 0 and 60th seconds. Reaction was initiated with the addition of 0.1 ml enzyme extract. Assessments were made by taking the change in absorbance within a minute into consideration (Jebara et al., 2005; Bagcı, 2010).

Superoxide dismutase (SOD) activity

SOD activity was determined through monitoring te inhibition of nitroblue tetrazolium (NBT) at 560 nm wave length. As a reaction solution, 50 mM Na-phosphate buffer (Na_2HPO_4 x H_2O_2), 0.1 mM Na- EDTA, 33 μM NBT, 5 μM riboflavin and 13 mM methionine mixture was used (pH: 7.0). About 2.5 ml reaction solution was mixed with 0.1 or 0.2 ml plant extract. Reaction was performed by keeping at 25 °C

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under 75 μmol m-2 s-1 (40 W) light for 10 min. Control solution without enzyme supplementation was kept at dark for the same duration. Readings were performed in control and reaction solution at 560 nm. SOD activity was identified as the activity reducing 50% of NTB (Rahnama and Ebrahimzadeh, 2005).

Ascorbate peroxidase (APX) activity

Ascorbate peroxidase (APX) activity was measured through ascorbic acid-induced inhibition of H_2O_2 at 290 nm. As the reaction solution, 50 mM phosphate buffer (KH_2PO_4), 0.5 mM ascorbic acid, 0.1 mM EDTA and 1.5 mM H_2O_2 mixture was used (pH: 7.0). About 3 ml reaction solution was mixed with 0.1 ml plant extract. Readings were performed in a spectrophotometer at 290 nm in 0 and 60th seconds. Reaction was initiated with 0.1 ml enzyme addition. Assessments were made by taking the changes in absorbance within a minute into consideration (Sairam et al., 2005).

Chlorophyll

0.25 g samples from the third leaf of the plant were homogenized in 80% acetone in a place where the light does not directly come out, then the extract completed to 25 ml with acetone after filtering. Prepared samples were read at 663 nm and 645 nm wavelength and calculated by the following formula ((Lichtenthaler, 1983; Amira, 2011).

$$\text{Chlorophyll a (mg/g)} = (12.7 * 663\ \text{nm}) – (2.69 * 645\ \text{nm}) * V / W*10000$$

$$\text{Chlorophyll b (mg/g)} = (22.91 * 645\ \text{nm}) – (4.68 * 663\ \text{nm}) * V / W*10000$$

$$\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Statistical analysis

Experiments were conducted in randomized plots – factorial experimental design. Experimental data were subjected to variance analysis and significant means were compared with Duncan's multiple range test (Yesilova and Denizhan, 2016). Statistical analyses were performed by using SAS 9.4 software (SAS, 2018).

Results

In the present study, quite significant differences were observed between the control plants and cold stress-treated plants. Cold stress reduced chlorophyll a, chlorophyll-b and total chlorophyll contents and such decreases were greater in sensitive genotypes than in tolerant genotypes (*Tables 1–3*). Under low temperature stress, the greatest chlorophyll a, chlorophyll-b and total chlorophyll contents were obtained from the 1st period of Ç30 common bean genotype (chlorophyll-a: 0.468 mg g^{-1} FW, chlorophyll-b: 0.188 mg g^{-1} FW, and total chlorophyll: 0.657 mg g^{-1} FW) and the lowest chlorophyll-a (0.206 mg g^{-1} FW), chlorophyll-b (0.106 mg g^{-1} FW) and total chlorophyll content (0.312 mg g^{-1} FW) were obtained from the 4th period of Ç13 genotype (*Tables 1–3*). Considering the effects of low temperature on total chlorophyll content, the greatest total chlorophyll content of 0.697 mg g^{-1} FW in the 1st period of low temperature-tolerant Ç30 bean genotype decreased to 0.578 mg g^{-1} FW in the 2nd period and

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decreased to 0.481 mg g^{-1} FW in the 4th period. In Yakutiye (Y) bean cultivar, total chlorophyll content of 0.616 mg g^{-1} FW in the 1st period decreased to 0.581 mg g^{-1} FW in the 2nd period and decreased to 0.460 mg g^{-1} FW in the 4th period. On the other hand, in low temperature-sensitive Ç13 bean genotype, total chlorophyll content of 0.633 mg g^{-1} FW in the 1st period decreased to 0.582 mg g^{-1} FW in the 2nd period and decreased to 0.312 mg g^{-1} FW in the 4th period. In low temperature-sensitive Zulbiye (Z) bean cultivar, total chlorophyll content of 0.631 mg g^{-1} FW in the 1st period decreased to 0.563 mg g^{-1} FW in the 2nd period and decreased to 0.326 mg g^{-1} FW in the 4th period (*Table 3*). In control plants, chlorophyll a, chlorophyll b and total chlorophyll contents increased in all 4 periods. The period-dependent differences in chlorophyll contents of the control and low temperature treatments were found to be significant (*Table 4*). Chlorophyll a+b values of the control treatments were identified as 0.731 mg g^{-1} FW for Ç30, 0.734 mg g^{-1} FW for Ç13, 0.727 mg g^{-1} FW for Yakutiye and 0.653 mg g^{-1} FW for Zulbiye. Chlorophyll a+b values of the low temperature treatments were identified as 0.562 mg g^{-1} FW for Ç30, 0.482 mg g^{-1} FW for Ç13, 0.541 mg g^{-1} FW for Yakutiye and 0.475 mg g^{-1} FW for Zulbiye (*Table 4*).

Table 1. *Change in chlorophyll a (mg g^{-1} FW) content of common bean genotypes under low temperature stress in 4 periods*

Genotype	Period 1 (day 25th)	Period 2 (day 30th)	Period 3 (day 35th)	Period 4 (day 40th)
Control				
Ç30	0.485d*	0.472de	0.508c	0.535ab
Yakutiye	0.460ef	0.480d	0.514c	0.534ab
Ç13	0.455eg	0.472de	0.522bc	0.541a
Zulbiye	0.356ı	0.418h	0.439	0.446fg
Low temperatures				
Ç30	0.468a*	0.412d	0.378e	0.337g
Yakutiye	0.439b	0.424cd	0.362f	0.326g
Ç13	0.458a	0.434bc	0.276h	0.206j
Zulbiye	0.456a	0.416d	0.255ı	0.217j

*Means in control and low temperature followed by the same letter are not significantly different ($P < 0.05$)

Table 2. *Change in chlorophyll b (mg g^{-1} FW) content of bean (Phaseolus vulgaris) genotypes under low temperature stress in 4 periods*

Genotype	Period 1 (day 25th)	Period 2 (day 30th)	Period 3 (day 35th)	Period 4 (day 40th)
Control				
Ç30	0.212g*	0.224ef	0.234cd	0.251a
Yakutiye	0.209g	0.221f	0.241bc	0.249ab
Ç13	0.223f	0.232ce	0.243ab	0.247ab
Zulbiye	0.233ce	0.228df	0.243ab	0.246ab
Low temperatures				
Ç30	0.188a*	0.168c	0.149de	0.143e
Yakutiye	0.176b	0.154d	0.148de	0.134f
Ç13	0.176b	0.147de	0.124g	0.106h
Zulbiye	0.175b	0.147de	0.125g	0.108h

*Means in control and low temperature followed by the same letter are not significantly different ($P < 0.05$)

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Table 3. *Change in chlorophyll a + b (mg g^{-1} FW) content of bean (Phaseolus vulgaris) genotypes under low temperature stress in 4 periods*

Genotype	Period 1 (day 25th)	Period 2 (day 30th)	Period 3 (day 35th)	Period 4 (day 40th)
Control				
Ç30	0.697de*	0.696df	0.742c	0.787a
Yakutiye	0.669g	0.701de	0.756bc	0.784a
Ç13	0.678fg	0.705d	0.765b	0.788a
Zulbiye	0.589ı	0.647h	0.682eg	0.693df
Low temperatures				
Ç30	0.657a*	0.581d	0.528f	0.481h
Yakutiye	0.616c	0.578d	0.511g	0.460ı
Ç13	0.633b	0.582d	0.401j	0.312m
Zulbiye	0.631b	0.563e	0.379k	0.326l

*Means in control and low temperature followed by the same letter are not significantly different ($P < 0.05$)

Table 4. *Change in chlorophyll a, b and chlorophyll a+b content of control and low temperature stress treatments*

Genotype	Chlorophyll a		Chlorophyll b		Chlorophyll a+b	
	Control	Low temperatures	Control	Low temperatures	Control	Low temperatures
Ç30	0.439d*	0.399a*	0.230b*	0.162a*	0.731a*	0.562a*
Yakutiye	0.461c	0.388b	0.230b	0.154b	0.727a	0.541b
Ç13	0.496b	0.343c	0.237a	0.138c	0.734a	0.482c
Zulbiye	0.514a	0.336d	0.238a	0.139c	0.653b	0.475c

*Means in each column followed by the same letter are not significantly different ($P < 0.05$)

There were differences in ion contents of the 1st and the 4th period and K, Ca and Mg contents decreased at the end of 4th period as compared to the 1st period. Such decreases were greater in low temperature sensitive bean plants (*Tables 5–7*). Under low temperature stress, the greatest K content (1.530%) was obtained from the 1st period of Zulbiye been genotype and the lowest K content (1.170%) was obtained from the 4th period of Ç13 bean genotype. The greatest Ca content (3.365%) was obtained from the 1st period of Zulbiye bean genotype and the lowest Ca content (1.645%) was obtained from the 4th period of Zulbiye bean genotype. The greatest Mg content (0.410%) was obtained from the 1st period of Zulbiye bean genotype, the lowest Mg content was obtained from the 4th period of again Zulbiye bean genotype (*Tables 6–7*).

As compared to the 1st period, plant K, Ca and Mg ion contents of the control groups increased at the end the 4th period. The K contents of Ç30, Yakutiye, Zulbiye and Ç13 genotypes were found to be significant under low temperature stress. The K content of 1.467% in the 1st period of Ç30 genotype decreased to 1.420% in the 2nd period, but placed in the same statistical group as Yakutiye (Y) cultivar with a K value of 1.442%. K contents also decreased in the 3rd and 4th periods, Ç30 and Zulbiye (Z) bean cultivar were placed in the same statistical group. K content of Ç30 bean genotype decreased to

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1.263% in the 4th period and K content of Zulbiye cultivar of the same statistical group decreased to 1.275%. The decreases in K content of Yakutiye bean cultivar in the 2nd and 3rd periods were not found to be significant (*Table 5*).

Table 5. *Change in potassium (K) content (%) of common bean genotypes under low temperature stress in 4 periods*

Genotype	Period 1 (day 25th)	Period 2 (day 30th)	Period 3 (day 35th)	Period 4 (day 40th)
Control				
Ç30	3.370c*	3.382c	3.407b	3.44a.
Yakutiye	3.265fg	3.282ef	3.292e	3.30e
Ç13	3.337d	3.365c	3.265fg	3.415b
Zulbiye	3.222ı	3.132j	3.237hı	3.257gh
Low temperatures				
Ç30	1.467c*	1.420d	1.335f	1.263g
Yakutiye	1.530b	1.442d	1.425d	1.335f
Ç13	1.422d	1.367e	1.232h	1.170ı
Zulbiye	1.567a	1.510b	1.263g	1.275g

*Means in control and low temperature followed by the same letter are not significantly different ($P < 0.05$)

With regard to Ca content of bean genotypes under low temperature stress, Ç30 genotype and Yakutiye (Y) bean cultivar were placed in the same statistical group in the 1st period of stress (Ç30: 3.475%; Y: 3.502%). Ç13 genotype and Zulbiye (Z) bean cultivar were also placed in the same statistical group (Ç13: 3.665%; Z: 3.647%). There were decreases in Ca contents of the bean genotypes also in the 2nd period of the low temperature stress. Ç30, Ç13 and Yakutiye bean genotypes were placed in the same statistical group. As compared to the 1st and 2nd periods, Ca contents decreased in the 3rd period (Ç30: 2.842%; Y: 3.357%; Ç13: 2.900%; Z: 2.695%) and in the 4th period (Ç30: 2.635%; Y: 2.785%; Ç13: 1.865%; Z: 1.645%) (*Table 6*).

Table 6. *Change in calcium (Ca) content (%) of bean (Phaseolus vulgaris) genotypes under low temperature stress in 4 periods*

Genotype	Period 1 (day 25th)	Period 2 (day 30th)	Period 3 (day 35th)	Period 4 (day 40th)
Control				
Ç30	3.562hi*	3.512k	3.612e	3.657cd.
Yakutiye	3.640d	3.677c	3.705b	3.522jk
Ç13	3.583fh	3.573gh	3.542ıj	3.593eg
Zulbiye	3.603ef	3.750a	3.575gh	3.462l
Low temperatures				
Ç30	3.475ac*	3.405bc	2.842de	2.635f
Yakutiye	3.502ac	3.400bc	3.357c	2.785df
Ç13	3.665a	3.565ab	2.900d	1.865g
Zulbiye	3.647a	3.400bc	2.695ef	1.645h

*Means in control and low temperature followed by the same letter are not significantly different ($P < 0.05$)

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While the differences in magnesium (Mg) contents of Ç30, Y and Z bean (*Phaseolus vulgaris*) genotypes were not found to be significant in the 1st period (Ç30: 0.362%; Y: 0.380%; Z: 0.382%) and in the 2nd period (Ç30: 0.262%; Y: 0.280%; Z: 0.285%) and they were placed in the same statistical group, but Ç13 genotype was found to be significantly different from the other groups. Magnesium content of genotypes decreased in the 3rd period, but all genotypes were placed in the same statistical group. In the 4th period of low temperature stress, decrease in Mg contents of Ç30, Ç13 and Y bean (*Phaseolus vulgaris*) genotypes (Ç30: 0.195%; Y: 0.195%; Ç13: 0.190%) were not found to be significant and they were all placed in the same statistical group, but Zulbiye (Z) bean genotype with an Mg content of 0.182% was found to be significantly different from the other genotypes (*Table 7*). Period-dependent differences in K, Ca and Mg contents of control and low temperature treatments were found to be significant (*Table 8*). K contents of control groups were identified as Ç30: 3.41%, Ç13: 3.34%, Yakutiye: 3.29% and Zulbiye: 3.21% and K contents of low temperature treatments were identified as Ç30: 1.37%, Ç13: 1.29%, Yakutiye: 1.43% and Zulbiye: 1.40% (*Table 8*).

Table 7. *Change in magnesium (Mg) content (%) of bean (Phaseolus vulgaris) genotypes under low temperature stress in 4 periods*

Genotype	Period 1 (day 25th)	Period 2 (day 30th)	Period 3 (day 35th)	Period 4 (day 40th)
Control				
Ç30	0.407dh*	0.435ce	0.510a	0.382fh
Yakutiye	0.385eh	0.422df	0.500a	0.365h
Ç13	0.397dh	0.447bd	0.475ac	0.390eh
Zulbiye	0.367gh	0.417dg	0.485ab	0.305ı
Low temperatures				
Ç30	0.362b*	0.262d	0.227e	0.195f
Yakutiye	0.380b	0.280d	0.230e	0.195f
Ç13	0.410a	0.310c	0.222e	0.190f
Zulbiye	0.385b	0.285d	0.237e	0.185f

*Means in control and low temperature followed by the same letter are not significantly different ($P < 0.05$)

Table 8. *Change in K (%), Ca (%) and Mg (%) content of control and low temperature stress treatments*

Genotype	K		Ca		Mg	
	Control	Low temperatures	Control	Low temperatures	Control	Low temperatures
Ç30	3.41a*	1.37c*	3.59b*	3.09b*	0.433ac*	0.261b*
Yakutiye	3.29c	1.43a	3.63a	3.26a	0.418a	0.271b
Ç13	3.34b	1.29d	3.57c	2.99c	0.427a	0.283a
Zulbiye	3.21d	1.40b	3.59b	2.85d	0.393b	0.273ab

*Means in each column followed by the same letter are not significantly different ($P < 0.05$)

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Significant differences were observed in MDA contents of the bean plants in 4 periods and increases were observed at the end of 4th period as compared to the 1st period. Such increases were greater in low temperature sensitive bean genotypes (*Table 9*). Similar changes were also observed in CAT, SOD and APX antioxidative enzyme activities. Increases were observed in CAT, SOD and APX activities of the bean genotypes in 4 periods. Increases were greater in the 4th period as compared to the 1st period and increases were also greater in low temperature-sensitive bean genotypes (*Tables 10–13*).

Table 9. *Change in malondialdehyde (MDA nmol g^{-1} FW) content of bean (Phaseolus vulgaris) genotypes under low temperature stress in 4 periods*

Genotype	Period 1 (day 25th)	Period 2 (day 30th)	Period 3 (day 35th)	Period 4 (day 40th)
Control				
Ç30	5.662b*	5.663b	5.713ab	5.735ab.
Yakutiye	5.691b	5.643b	5.719ab	5.732ab
Ç13	5.634b	5.899a	5.786ab	5.813ab
Zulbiye	5.662b	5.615b	5.767ab	5.799ab
Low temperatures				
Ç30	5.613l*	5.925h	5.954g	6.106e
Yakutiye	5.685j	5.881ı	5.981g	6.114e
Ç13	5.646k	6.057f	6.249c	6.325b
Zulbiye	5.668jk	6.217d	6.255c	6.373a

*Means in control and low temperature followed by the same letter are not significantly different ($P < 0.05$)

Table 10. *Change in superoxide dismutase (SOD unit g^{-1} FW) content of bean (Phaseolus vulgaris) genotypes under low temperature stress in 4 periods*

Genotype	Period 1 (day 25th)	Period 2 (day 30th)	Period 3 (day 35th)	Period 4 (day 40th)
Control				
Ç30	105.025fh*	107.158cg	109.449c	118.930b
Yakutiye	107.980cf	104.642gh	109.248cd	118.768b
Ç13	103.910h	106.278dh	109.200cd	121.815a
Zulbiye	103.884h	105.388eh	108.235ce	122.059a
Low temperatures				
Ç30	102.226e*	116.015d	121.513c	144.875b
Yakutiye	101.283e	116.510d	119.986dc	146.259b
Ç13	102.829e	124.475c	122.594c	157.812a
Zulbiye	102.738e	124.662c	124.193c	157.873a

*Means in control and low temperature followed by the same letter are not significantly different ($P < 0.05$)

Despite the insignificant differences in malondialdehyde (MDA) activity of the control groups in the 1st and 2nd periods, the MDA activity of Ç13 genotype was

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identified as 5.899 nmol g^{-1} FW in the 2nd period. Significant increases were observed in MAD activities of the bean genotypes in the first two periods of low temperature stress (*Table 9*). The increase in MAD activity continued in the 3rd period, Ç30 (5.954 nmol g^{-1} FW) and Y (5.981 nmol g^{-1} FW) genotypes were placed in the same statistical group, Ç13 (6.248 nmol g^{-1} FW) and Z (6.255 nmol g^{-1} FW) were also placed in the same statistical group (*Table 7*). Such increases also continues in the 4th period of low temperature stress, Ç30 bean genotype (6.106 nmol g^{-1} FW) and Y bean cultivar (6.114 nmol g^{-1} FW) were placed in the same statistical group, Ç13 genotype had an MDA activity of 6.325 nmol g^{-1} FW and Zulbiye bean cultivar had an MDA activity of 6.373 nmol g^{-1} FW (*Table 9*).

Table 11. *Change in catalase (CAT mmol g^{-1}) content of bean (Phaseolus vulgaris) genotypes under low temperature stress in 4 periods*

Genotype	Period 1 (day 25th)	Period 2 (day 30th)	Period 3 (day 35th)	Period 4 (day 40th)
Control				
Ç30	0.176d*	0.186c	0.193bc	0.196b
Y	0.175d	0.184c	0.193b	0.197b
Ç13	0.171e	0.186c	0.194 b	0.210a
Z	0.167f	c0.185h	0.196b	0.214a
Low temperatures				
Ç30	0.172h*	0.184f	0.203df	0.217c
Y	0.176gh	0.187f	0.203df	0.224b
Ç13	0.178g	0.193e	0.216c	0.244a
Z	0.176g	0.186f	0.215c	0.245a

*Means in control and low temperature followed by the same letter are not significantly different ($P < 0.05$)

Table 12. *Change in ascorbate peroxidase (APX mmol g^{-1} FW) content of bean (Phaseolus vulgaris) genotypes under low temperature stress in 4 periods*

Genotype	Period 1 (day 25th)	Period 2 (day 30th)	Period 3 (day 35th)	Period 4 (day 40th)
Control				
Ç30	0.733l*	0.759ıj	0.781f	0.812d
Yakutiye	0.755j	0.763gh	0.793e	0.826c
Ç13	0.747k	0.0.766g	0.778f	0.855a
Zulbiye	0.756j	0.771g	0.791e	0.836b
Low temperatures				
Ç30	0.759i*	0.766h	0.903e	0.928d
Yakutiye	0.731k	0.771h	0.873f	0.923d
Ç13	0.747j	0.784g	1.124b	1.162a
Zulbiye	0.742j	0.785g	1.116c	1.167a

*Means in control and low temperature followed by the same letter are not significantly different ($P < 0.05$)

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Table 13. *Change in MDA (nmol g^{-1} FW), CAT (mmol g^{-1} FW), SOD (unit g^{-1} FW) and APX (mmol g^{-1} FW) content of control and low temperature stress treatments*

Genotype	MDA		CAT		SOD		APX	
	Control	Low temperatures	Control	Low temperatures	Control	Low temperatures	Control	Low temperatures
Ç30	5.68b*	5.89d*	0.188b	0.194d*	110.14a*	121.16b*	0.771c*	0.839b*
Yakutiye	5.69ab	5.92c	0.0.187b	0.197c	110.16a	121.01b	0.784b	0.824c
Ç13	5.78a	6.07b	0.190a	0.208a	110.31a	127.17a	0.786ab	0.954a
Zulbiye	5.71ab	6.13a	0.190a	0.205b	109.89a	127.36a	0.788a	0.952a

*Means in each column followed by the same letter are not significantly different ($P < 0.05$)

The changes in superoxide dismutase (SOD) activity of the bean genotypes under low temperature stress were not found to be significant in the 1st period and they were all placed in the same statistical group. In the 2nd period, Zulbiye (124.662 unit g^{-1} FW) and Ç13 (124.475 unit g^{-1} FW) bean genotypes were placed in the same statistical group and Yakutiye (116.510 unit g^{-1} FW) and Ç30 (116.015 unit g^{-1} FW) bean genotypes were placed in the same statistical group. In the 4th period of low temperature stress, greater increases were observed in the SOD activities of Ç13 (157.812 unit g^{-1} FW) and Zulbiye (157.873 unit g^{-1} FW) genotypes (*Table 10*). With regard to catalase (CAT) activity in the 1st period of low temperature stress, Ç13 (0.178 mmol g^{-1} FW) and Zulbiye (0.176 mmol g^{-1} FW) bean genotypes were placed in the same statistical group, but Yakutiye (0.176 mmol g^{-1} FW) and Ç30 (0.172 mmol g^{-1} FW) bean genotypes were significantly different from the other genotypes. A slight increase was observed in CAT activities of the genotypes in the 2nd period of low temperature stress, but differences in CAT activities of the genotypes, except for Ç13, were not found to be significant. In the 3rd period of stress, Ç30 and Yakutiye genotypes had a CAT activity of 0.203 mmol g^{-1} FW, Ç13 genotypes had a CAT activity of 0.216 mmol g^{-1} FW and Zulbiye genotype had a value of 0.215 mmol g^{-1} FW. The differences among the genotypes were not significant, thus they were all placed in the sme statistical group. In the 4th period of low temperature stress, the differences in CAT activities of the genotypes (Ç30: 0.217 mmol g^{-1} FW; Y: 0.224 mmol g^{-1} FW; Ç13: 0.244 mmol g^{-1} FW; Z: 0.245 mmol g^{-1} FW) were found to be significant (*Table 11*). With regard to ascorbate peroxidase (APX) activity of the 1st period, Ç13 (0.747 mmol g^{-1} FW) and Zulbiye (0.742 mmol g^{-1} FW) bean genotypes were placed in the same statistical group, thus the difference between them were not significant. Yakutiye (0.731 mmol g^{-1} FW) and Ç30 (0.759 mmol g^{-1} FW) bean genotypes were found to be significantly different from the other genotypes. In the 2nd period of stress, there was a slight increase in APX activity of the sensitive and tolerant genotypes and the differences in APX activity of sensitive and tolerant genotypes were found to be significant. There were significant differences in APX activity of the genotypes in the 3rd (Ç30: 0.903 mmol g^{-1} FW; Y: 0.873 mmol g^{-1} FW; Ç13: 1.124 mmol g^{-1} FW; Z: 1.116 mmol g^{-1} FW) and 4th (Ç30: 0.928 mmol g^{-1} FW; Y: 0.923 mmol g^{-1} FW; Ç13: 1.162 mmol g^{-1} FW; Z: 1.167 mmol g^{-1} FW) period of low temperature stress (*Table 12*).

Period-dependent differences in MDA, CAT, SOD and APX activity of control and low temperature treatments were found to be significant. Low temperature-tolerant Yakutiye cultivar had the values of MDA: 5.69 nmol g^{-1} FW, CAT: 0.187 mmol/g FW,

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SOD: 110.16 unit g^{-1} FW, APX: 0.784 mmol g^{-1} FW in control treatments and had the values of MDA: 5.92 nmol g^{-1} FW, CAT: 0.197 mmol g^{-1} FW, SOD: 121.01 unit g^{-1} FW, APX: 0.824 mmol g^{-1} FW in low temperature treatments. Low temperature-sensitive Zulbiye cultivar had the values of MDA: 5.71 nmol g^{-1} FW, CAT: 0.190 mmol g^{-1} FW, SOD: 109.89 unit g^{-1} FW, APX: 0.788 mmol g^{-1} FW in control treatments and had the values of MDA: 6.13 nmol g^{-1} FW, CAT: 0.205 mmol g^{-1} FW, SOD: 127.36 unit g^{-1} FW, APX: 0.952 mmol g^{-1} FW in low temperature treatments.

Significant findings were obtained for investigated parameters of present study both in control and low temperature stress treatments. Among the investigated parameters, decreases were observed in chlorophyll and ion contents of the genotypes in 4 periods and increases were observed in MDA, CAT, SOD and APX activity of the genotypes. The differences between the genotypes and between the periods were found to be significant.

Discussion

Some significant recession is observed in plant growth and development under low temperatures. This recession is greater in low temperature-sensitive genotypes. In the present study, changes in chlorophyll, K, Ca, and Mg contents and lipid peroxidation (MDA), catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities of low temperature-tolerant and sensitive bean (*Phaseolus vulgaris*) genotypes were investigated in four different periods of low temperature stress. Present findings were quite similar with the findings of the earlier studies.

There were decreases in chlorophyll a, chlorophyll b and total chlorophyll contents of tolerant and sensitive genotypes in four periods *(Tables 1–3*). Such decreases were greater in low temperature sensitive Ç13 and Zulbiye (Z) genotypes than in low temperature-tolerant Ç30 and Yakutiye (Y) bean genotypes. There were also significant differences in investigated parameters of the control and low temperature treatments of four periods (*Table 4*). Decreases were reported in chlorophyll a, chlorophyll b and total chlorophyll contents of bean and tomato plants under abiotic stress conditions (Terzi et al., 2010; Kabay and Şensoy, 2016, 2017; Alp and Kabay, 2017). Similar findings were reported in literature about the effects of different low temperatures. It was reported in a previous study investigating the effects of low temperature stress on physiological characteristics of tomato genotypes that cold stress reduced chlorophyll and dry matter content of young and old leaves (Gökmen, 2006). Low temperatures increased chlorophyll and weight losses of cabbages (Soengas et al., 2018). Cold stress decreased photosynthetic processes of tea leaves, but melatonin treatments reduced antioxidant potential and oxidative stress and increased photosynthetic capacity (Li et al., 2018). Low temperatures (14/12 °C) altered osmatic potential of maize leaf cells and ultimately altered cell membrane composition (Bilska-kos et al., 2017).

In control groups, significant increases were observed in potassium (K), calcium (Ca) and Magnesium (Mg) content of bean (*Phaseolus vulgaris*) genotypes in four periods. The changes in K, Ca and MG content of bean genotypes under low temperature stress were also found to be significant and K, Ca and Mg contents decreased in four periods. Such decreases were greater in stress-sensitive genotypes (*Tables 5–7*). The decreases in K content of Yakutiye (Y) bean cultivar in the 2nd and 3rd periods were not found to be significant. The decreases in K content of cv. Zulbiye in the 3rd and 4th periods were also not found to be significant (*Table 5*). The changes in Ca and Mg contents were

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found to be significant in the 4th period of stress (*Tables 6* and *7*). Throughout 4 periods, changes in K, Ca and Mg content of bean genotypes in control and low temperature treatments were found to be significant and ion contents decreased under low temperature stress (*Table 8*).

It was reported that low temperature stress negatively influenced leaf cells of maize plants (Bilska-kos et al., 2017). Low soil temperatures in spring negatively influence seedling growth and development of sensitive bean plants (Elkoca et al., 2005). Low temperature stress was reported to have negative effects on cold stress-resistant and sensitive sugar cane cultivars (Sun et al., 2017). It was reported in study carried out to determine total temperature demands of bean plants at different growth stages that the greatest yield was obtained from early July sowings and yields decreased as the sowing time approached to autumn (Yoldaş and Eşiyok, 2009). High and low temperatures (15/8, 20/13, 28/21, 33/23 and 36/26 °C) were applied to tomato plants and it was observed that temperature stress negatively influenced plant growth and development and nutrient uptake (Sung et al., 2015). In vegetables, low temperatures at root zone generate a stress in water absorption of the roots (Sakamoto and Suzuki, 2015). Decreasing chlorophyll, K and Ca contents were reported for bean and tomato plants under abiotic stress (Terzi et al., 2010; Kabay and Şensoy, 2016; Zhou et al., 2017; Uzal and Yasar, 2017; Kabay et al., 2017; Alp and Kabay, 2017)

Significant differences were observed in MDA contents of the bean plants in 4 periods and increases were observed at the end of 4th period as compared to the 1st period. Such increases were greater in low temperature sensitive bean genotypes (*Table 7*). Similar changes were also observed in CAT, SOD and APX antioxidative enzyme activities. Increases were observed in CAT, SOD and APX activities of the bean genotypes in 4 periods. Increases were greater in the 4th period as compared to the 1st period and increases were also greater in low temperature-sensitive bean genotypes (*Table 9–12*). With regard to MDA activity, there were significant differences both between the bean genotypes and between the stress periods (*Table 9*). There were significant differences in superoxide dismutase (SOD) activity of the bean genotypes in the 4th period of stress. However, the differences in SOD activity of Ç13 and Zulbiye genotypes were not significant different in the 2nd and 3rd period of the stress (*Table 10*). There were significant differences in catalase (CAT) activity of both the bean genotypes and the stress periods (*Table 11*). The changes in CAT activity of Ç30 and Yakutiye bean genotypes were not found to be significant in 2nd and 3rd period of stress (*Table 11*). The differences in ascorbate peroxidase (APX) activity of both the genotypes and the stress periods were also found to be significant (*Table 12*). Both the control and the low temperature treatments yielded significant differences in MDA, CAT, SOD and APX activity of the bean genotypes. Significant increases were observed in MDA, CAT, SOD and APX activities under low temperature stress (*Table 13*). Xintaimici and Jinyan cucumber (*Cucumis sativus* L.) cultivars were subjected to two different temperature regimes (15/15 °C and 25/18 °C) under low light intensity and significant increases were reported in leaf superoxide dismutase and less increases were reported in leaf catalase activity (Xu et al., 2008). Increasing ascorbate peroxidase (APX) and superoxide dismutase (SOD) activities of melon and pumpkin plants were reported under low temperature conditions (Do et al., 2018).

In process of cold resistance, plants develop different mechanisms against cold stress-induced damages and ultimate die outs or develop such mechanisms to provide cold-tolerance such as physical changes in cell membranes, changes in some soluble

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substances in plant extract, lipid composition of cell membranes, protein contents, enzyme activity and antioxidant systems and plant nutrients (Aslantaş et al., 2010). It was reported in a previous study investigating the effects of low temperature stress on physiological characteristics of tomato genotypes that young and old leaves of the plants were found to be insufficient in antioxidative systems (Gökmen, 2006). Low temperatures were reported to increase antioxidant enzyme and MDA content of rapeseed plants (Xian et al., 2017). Low temperatures increased chlorophyll and weight losses of cabbages and increased reactive oxygen species (Soengas et al., 2018). In a previous study carried out with cold stress resistant and sensitive sugar cane cultivar, cell structure was destructed and cold stress-induced plants had greater MDA, proline, soluble sugar, soluble protein, POD and SOD activity than the control plants (Sun et al., 2017). It was reported in another study investigating the effects of cold stress on rye and wheat seedlings that cold stress increased antioxidant enzyme activities, proline, sugar and anthocyanins contents of both species (Kolupaev et al., 2016). Significant decreases were reported in malondialdehyde, proline, peroxidase and catalase activities of control and transgenic tomato plants under cold and low temperature stress (Yu et al., 2015). While significant increases were reported in antioxidant compound quantities of tomato plants, decreases were reported in chlorophyll contents (Gökmen, 2006). In present study, significant decreases were observed in chlorophyll, K, Ca and Mg contents and increases were observed in MDA, CAT, SOD and APX activities of bean genotypes under low temperature conditions. Present findings were mostly well complied with the results of similar earlier studies.

Conclusion

Significant differences were observed in chlorophyll contents, enzyme activities and ion contents of low temperature-resistant and sensitive common bean (*Phaseolus vulgaris*) genotypes throughout cold stress treatments. The decrease in chlorophyll-a, chlorophyll-b, total chlorophyll, K, Ca and Mg contents were greater in low temperature-sensitive bean plants than in low temperature-tolerant plants.

With the progress of cold stress, the MDA content of sensitive genotypes were greater than the MAD contents of tolerant genotypes. On the other hand, tolerant genotypes had greater CAT, SOD and APX antioxidant enzyme activities than the sensitive genotypes. Present findings proved one more time the suitability of the parameters selected to determine the effects of low temperature stress for selection of drought stress-tolerant bean genotypes.

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