Research Article

Effects of Cold Stress on Enzyme Activities in Peas§

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Abstract
This research was conducted in three replications according to the "Two Factor Factorial Experiment in Random Plots" design and Selcuk University, Faculty of Agriculture, Department of Field Crops, in a fully controlled research greenhouse and laboratories in 2015. Pea genotypes resistant to cold stress were determined, and biochemical, physical properties or physical defense mechanisms created by plants against cold stress were sought. According to the results of the research, if cold harm was examined, Melrose, Sahin, Granger, 4053 x Melrose, 4053 x Hadim, Sahin x Hadim and 3057 x Melrose; if peroxidase content was examined 3057 x Melrose, 4053 x Melrose, 3029 x Melrose, 3029 x Granger and 4053 x Hadim, when superoxide content was examined, 3053 x Melrose, Sahin x Hadim, 4053 x Melrose, Sahin x Melrose and 3029 x Melrose and when proline content examined 3031 x Granger, 3055 x Melrose, Ultrillo, 3057 x Hadim and Sahin x Hadim the genotypes were first. As a result, when the effects of cold stress on the enzyme activities in the leaves of pea genotypes are considered, the most durable genotypes are 3031 x Granger and 3055 x Melrose. These come forward as genotypes that can be used in subsequent studies to breed for cold resistance.

Key words: Cold stress, Cold Reaction, POX, Proline, SOD.

Bezelyede Enzim Aktiviteleri Üzerine Soğuk Stresinin Etkileri

Özet
Araştırma “Tesadüf Parsellerinde iki Faktörü Faktöriyel Deneme” desenine göre üç tekerrürlü olarak kurulmuştur ve Selçuk Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü tam kontrollü araştırma serası ve laboratuvarlarlarında 2015 yılında yürütülmüştür. Bu araştırma ile soğuk stresine dayanıklı uygun bezelye genotipleri belirlenmiştir. Ayrıca soğuk stresine karşı bitkiler tarafından oluşturulan biyokimyasal, fiziksel özellikler veya fiziksel savunma mekanizmaları ortaya konulmamıştır. Araştırma sonuçlarına göre; soğuk zararı bakımından Melrose, Sahin, Granger, 4053 x Melrose, 4053 x Hadim, Şahin x Hadim ve 3057 x Melrose, peroksidad içeriği incelendiğinde 3057 x Melrose, 4053 x Melrose, 3029 x Melrose, 3029 x Granger ve 4053 x Hadim, süperoksid dismutaz içeriği incelendiğinde 3053 x Melrose, Şahin x Hadim, 4053 x Melrose, Şahin x Melrose ve 3029 x Melrose ve prolin içeriği incelendiğinde 3031 x Granger, 3055 x Melrose, Ultrillo, 3057 x Hadim ve Şahin x Hadim genotipleri ilk sıralarda yer almıştır. Sonuç olarak, soğuk stresinin bezelye genotiplerinin yapraklarında enzim aktiviteleri üzerine etkileri ele alındığına; genotipler içerisinde en dayanıklı olarak 3031 x Granger ve 3055 x Melrose genotipleri ön plana çıkan bu genotipler daha sonra yapılabacak olan soğukta dayanıklılık islah çalışmalarında kullanılabilir.

Anahtar kelimeler: POX, Prolin, Soğuga Tepki, Soğuk Stresi, SOD.

Introduction
As is every plant, the pea is exposed to various biotic (pathogens, competition with other organisms etc.) and abiotic (drought, salinity, radiation, high temperature or frost, etc.) stress factors over its lifetime. These stresses lead to changes in the physiological functions of peas (Lichtenhale, 1996). In many parts of the world
where pea cultivation is carried out, as in all plants, it is affected by extreme temperatures, which is an abiotic stress factor in peas (Bruggemann et al., 1995; Saltweil, 2001). For this, pea plants developed physiological and biochemical strategies against extreme environmental conditions (Nilsen and Orcutt, 1996). Low-temperature exposure in plants affects vital cycles such as germination, growth, development, reproductive organs and post-harvest storage time (Wang, 1990). In this case, plants develop their own mechanisms and develop antioxidant defense systems against cold stress (Yang et al., 2001; Tasgın et al., 2003; Posmyk et al., 2005). In addition, the cell membrane and organelles resist the harmful formation of reactive oxygen species (ROS) with antioxidant defense systems (Lee and Lee, 2000). The most important of these antioxidant defense systems are proline, superoxide dismutase (SOD) and peroxidase (POX). Proline acts as an enzyme protector to provide osmotic membrane integrity and plays a role in removing reactive oxygen species (Ozturk and Demir, 2002; Nayyar et al., 2005). SOD is present in the cells of all aerobic organisms and catalyses the superoxide radical as hydrogen peroxide (Møller, 2001). In plants, increased SOD can oppose the oxidative stress caused by abiotic stress and plays an important role in that by going on their viability under stress conditions (Duman et al., 2016). POX is present in leaves, damaged stems, cotyledon leaves and flower stalks and is found to be localized in nuclei, mitochondria, ribosomes, cell membranes and extracellular regions (Bergmeyer and Grabl, 1983; Banci, 1997; Kim et al., 2000; Tasgın et al., 2003; Mutlu et al., 2009). On plants, POX lignification, oxidation of phenolics and regulation of cell elongation and is actively involved in the detoxification of toxic compounds such as H$_2$O$_2$ that arise as a result of oxidative stress (Scebbba et al., 1998). Previous authors found that plants increased the amount of SOD, POX and proline in their structures when exposed to cold stress (Scebbba et al., 1998; Atıcı and Nalbantoğlu, 1999; Lee and Lee, 2000; Ozturk and Demir, 2002; Nayyar et al., 2005). In this study, we attempted to determine the changes that occur in the amount of antioxidant enzymes (SOD, POX and proline) when a pea plant is exposed to cold stress.

**Material and Methods**

In this study, two cold-tolerant pea varieties (Sahin and Melrose) and one very cold-sensitive variety (Ultrillo) were examined. In all, 25 F$_2$ populations obtained from hybrids of these two varieties were used as materials. The seeds of the varieties and populations used for the research were treated with 5% sodium hypochlorite for 10 minutes and then sterilized by washing 3 times with deionized water. Then, before sowing, to this variety and populations seeds belong to were washed 14 x 13 cm size and planted in sterilized pots with 1 kg of soil and peat mixture. Three replicates were set up according to ‘Coincidence Parcels Experimental Plots’. The pots were covered after sowing and then kept in a fully controlled greenhouse (25°C, 50% humidity) for 7 days. After seed germination, the pots were uncovered, and the seedlings grown in a fully controlled greenhouse for 2 weeks. After this process, the plants were kept in a fully controlled growth cabinet (capable of operating at temperatures between -25°C and 45°C) for 2 weeks at 4°C. Respectively, first minimum temperature applications were made. The temperature in the cabinet was reduced gradually at a rate of 2°C per hour until it reached -8°C and -12°C. Once the temperatures reached -8°C and -12°C, the plants were allowed to stand for one hour, and then the temperature was increased by 2°C per hour to 4°C. When the temperature reached 4°C, the plants were transferred to a fully controlled greenhouse. Each of these processes were carried out separately. Samples for enzyme analysis were obtained at -8°C and -12°C. At the end of 1 week, cold damage in plants was assessed on a scale of 1-9 (Fiebelkorn, 2013). Control and cold stress made application SOD, POX separately for the preparation of enzyme analysis extracts was weighed 0.5 g. For proline, 0.1 g pea leaf samples were taken, flash frozen in liquid nitrogen and stored at -80°C. Samples (0.5 g) of pea leaves were removed from the freezer and ground with a chilled pestle in 2% w/v polyvinylpolypyrrolidone (PVPP) in liquid nitrogen. Samples were homogenized with 50 mM Na-phosphate buffer, pH 7.8, containing 1 mM EDTA, filtered at + 4°C and centrifuged at 14 000 rpm for 30 minutes. This process was applied separately for each antioxidant enzyme: POX (EC 1.11.1.7), SOD (EC 1.15.1.1) and proline. The whole extraction procedure was performed at ± 4°C. POX enzyme analysis was performed according to the method of Kumar and Khan (1982). The mixture used for the determination of POX consisted of 2 ml of 0.1 M phosphate buffer (pH = 6.8) solution, 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H$_2$O$_2$ and 0.5 ml of enzyme extract. To this prepared solution was added 1 ml of 2.5 M H$_2$SO$_4$, and the mixture was incubated at 25°C for 5 min. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm (Kumar and Khan, 1982; Gökmen and Ceyhan, 2015). Enzyme activity was expressed as units mg$^{-1}$ protein.
The method of Beauchamp and Fridovich (1971) was used to assay the enzyme SOD. A reaction mixture containing 1.17 M riboflavin, 0.1 M methionine, 2 x 10^{-5} M KCN and 5.6 x 10^{-3} M NBT salt 0.05 M sodium phosphate (pH = 7.8) buffer solution was allowed to dissolve in 3 ml. One milliliter of enzyme extract was added to the medium. The absorbance was read in a spectrophotometer at a wavelength of 560 nm (Beauchamp and Fridovich, 1971; Gokmen and Ceyhan, 2015). SOD activity was expressed as units mg^{-1} protein. To determine the free proline content, the method of Bates et al. (1973) was used. The absorbance of the toluene fraction aspirated from the liquid phase was read at 520 nm in a spectrophotometer. The proline concentration was calculated using the calibration curve and expressed as µmol proline g^{-1} fresh weight. The MSTAT-C package was used for these analyses and calculations. In this study, the values of the belong to investigated properties the "Two Factorial Factorial Trial in Random Plots "pattern according to was made analysis of variance and grouping was made by Lsd analysis for the features with statistical differences in between them (Duzgunes et al., 1987).

Table 1. Analysis of variance of cold damage scale belong to values of genotypes in cold stress.

| Variance Sources | SD  | Sum of Squares | Mean of Squares | F Value |
|------------------|-----|----------------|-----------------|---------|
| General          | 167 | 766.571        |                 |         |
| Stress Application(SU) | 2   | 466.667        | 233.333         | 1285.246** |
| Genotypes (G)    | 27  | 221.571        | 8.206           | 22.601** |
| SU x G Int.      | 27  | 37.667         | 0.698           | 3.842** |
| Fault            | 112 | 40.667         | 0.363           |         |

** : p < 0.01

Table 2. Cold stress genotypes average value of cold damage scale and LSD values (%).

| Genotypes              | -8°C Temperature | -12°C Temperature | Mean |
|------------------------|------------------|-------------------|------|
| Sahin                  | 2.000 mno        | 4.333 hij         | 3.167 jk |
| 4009 x Melrose         | 3.000 klm        | 6.333 def         | 4.667 d-h |
| Bolero x Melrose       | 3.667 ijk        | 7.333 bcd         | 5.500 cd |
| 3057 x Melrose         | 2.000 mno        | 6.000 ef          | 4.000 g-j |
| 4053 x Melrose         | 2.333 l-o        | 4.333 hij         | 3.333 ij |
| 3029 x Melrose         | 3.667 ijk        | 6.667 cde         | 5.167 cde |
| 3029 x Granger         | 2.667 k-n        | 5.667 efg         | 4.167 f-i |
| 3053 x Hadim           | 4.333 hij        | 6.667 cde         | 5.500 cd |
| 3048 x Melrose         | 4.333 hij        | 7.333 bcd         | 5.833 bc |
| Ultrillo              | 5.667 efg        | 8.667 a           | 7.167 a |
| 3053 x Melrose         | 3.667 ijk        | 6.667 cde         | 5.167 cde |
| Sahin x Hadim          | 2.333 l-o        | 5.333 fgh         | 3.833 hij |
| 3031 x Granger         | 3.667 ijk        | 6.667 cde         | 5.167 cde |
| 3053 x Ultrillo        | 5.667 efg        | 7.667 abc         | 6.667 ab |
| 3055 x Melrose         | 3.667 ijk        | 6.667 cde         | 5.167 cde |
| 4028 x Hadim           | 3.333 jkl        | 7.667 abc         | 5.500 cd |
| Melrose                | 1.333 o          | 3.333 jkl         | 2.333 k |
| 3057 x Granger         | 3.000 klm        | 6.667 cde         | 4.833 d-g |
| Sahin x Melrose        | 2.667 k-n        | 5.667 efg         | 4.167 f-i |
| 4053 x Hadim           | 2.000 mno        | 5.667 efg         | 3.833 hij |
| 3029 x Ultrillo        | 2.333 l-o        | 8.000 ab          | 5.167 cde |
| 3057 x Hadim           | 2.333 l-o        | 6.667 cde         | 4.500 e-h |
| Sahin x Ultrillo       | 6.333 def        | 8.667 a           | 7.500 a |
| 3048 x Ultrillo        | 3.667 ijk        | 6.667 cde         | 5.167 cde |
| 4028 x Melrose         | 2.667 k-n        | 7.333 bcd         | 5.000 c-f |
| Granger                | 1.667 no         | 4.667 ghi         | 3.167 jk |
| 3057 x Granger         | 2.667 k-n        | 7.667 abc         | 5.167 cde |
| 4028 x Granger         | 2.667 k-n        | 7.667 abc         | 5.167 cde |

Mean 3.190 6.524 4.857

Genotypes (G) LSD_{0.05}: 0.9115; SU x G Int. LSD_{0.05}: 1.289.
**Results and Discussion**

For cold stress the cold harm of genotypes results of variance analysis belonging to scale values are given (Table 1).

The differences between genotypes in terms of the scale values of cold damage were found to be statistically significant at 1% mean on probability of cold stress (Table 1). In this experiment, the lowest cold damage according to the mean of genotypes occurred between 3.190 and -8°C cold stress and the highest was between 6.524 and -12°C cold stress (Table 2). The mean scale value and Lsd test results for cold damage by genotype under cold stress are given in Table 2.

Many previous studies have shown that pea plants are affected by cold (Auld et al., 1983a; Auld et al., 1983b; Ettev, 1985; Bourion et al., 2003; Ceyhan, 2003). In this study, as the temperature decreased, greater cold damage occurred in peas. Pea plants were not greatly affected at -8°C but were damaged more by exposure to a temperature of -12°C. From this point onward, to test for cold harm, we believe that temperature applications of -8°C would be more appropriate.

According to the results of this research, the lowest cold harm of 2.333 was obtained in the Melrose genotype, and the highest the cold loss harm of 7.500 was obtained in the Sahin x Ultrillo genotype. Cold harm in other genotypes used in the study varied between these values. Melrose, Sahin, Granger, 4053 x Melrose, 4053 x Hadim, Sahin x Hadim and 3057 x Melrose genotypes were take place in the first place (Table 2). The majority of genotypes were obviously harmed by cold stress at -12°C, whereas, when cold stress was applied at -8°C, almost all genotypes survived and did not suffer much damage from the cold.

Resistance to cold in pea depends on the environmental conditions as well as the genotype. Environmental conditions change more or less every year. While in some years the weather in winter is unexpectedly mild for the region, in other years it is unexpectedly hard and very cold. In this respect, the execution of cold resistance tests of genotypes carries great importance for extreme cold years (Auld et al., 1983a; Auld et al., 1983b; Ettev, 1985; Bourion et al., 2003; Ceyhan, 2003). In this study, we applied a temperature of -12°C without snow cover, and genotypes based on this temperature application are extremely important.

The results of variance analysis of cold damage scale values (POX, SOD and proline) for the various genotypes are given. According to the results of variance analysis, differences between genotypes in terms of the POX, SOD and proline content were found to be statistically an important ratio 1% (Table 3). POX, SOD and proline contents and Lsd values of cold stress applications are given in Table 4.

**Table 3.** Cold stress applications genotypes peroxidase, superoxide dismutase and proline belong to analysis of variance of scale.

| Variance source   | SD  | Peroxidase (POX) | Superoxide dismutase (SOD) | Proline |
|-------------------|-----|------------------|----------------------------|---------|
| Stress application (SU) | 2   | 261.716**        | 897.736**                  | 3.362** |
| Genotypes (G)     | 27  | 22.198**         | 44.345**                   | 0.619** |
| SU x G Int.       | 54  | 4.556**          | 7.241**                    | 0.163** |
| Fault             | 168 | 0.151            | 0.040                      | 0.002   |

****: p < 0.01

**Examination of peroxidase content**

When the POX content was examined, the highest values for all genotypes were obtained with 11.050 units mg⁻¹ protein in the -8°C cold stress group, and the lowest POX content was obtained for the control group, with 7.532 units mg⁻¹ protein. According to the computed Lsd test, -8°C in cold stress to the first group (a), -12°C in cold stress was in the second group (b) and control was in the last group (c) (Table 4).

In previous studies, one of the reactions of many different plants to cold stress was that they accumulated large amounts of POX (Scebbba et al., 1998; Atıcı and Nalbantoğlu, 1999; Lee and Lee, 2000; Ozturk and Demir, 2002; Nayyar et al., 2005). In this study, it was observed that the POX contents of pea genotypes was greatly increased with cold stress. Our results are in close agreement with those of previous studies.

In this study, the highest POX content as the average of cold stresses was obtained for the 3029 x Melrose genotype, with 12,376 units mg⁻¹ protein, and the lowest POX content was determined for the 4028 x Granger genotype, with 7.040 units mg⁻¹ protein. The POX content of other genotypes included in the study were between these values. In this study, we observed that the POX content increased by more when cold stress was applied at -8°C. The genotypes used in the experiment included 3057 x Melrose, 4053 x Melrose, 3029 x Melrose, 3029 x Granger and 4053 x Hadim first place. Take part starting course genotypes.
| Genotype      | POX (%) | SOD (%) | Prolin (%) |
|--------------|---------|---------|------------|
| **Control**  | **-8°C** | **-12°C** | **Mean** |
| Sahin        | 6.651   | 9.207   | 8.826 ijk  |
| 4009 x Melrose | 6.817   | 8.819   | 7.533      |
| Bolero x Melrose | 9.219   | 11.303  | 5.250      |
| 3057 x Melrose | 9.037   | 12.830  | 6.057      |
| 4053 x Melrose | 9.753   | 16.782  | 7.167      |
| 3029 x Melrose | 7.388   | 16.270  | 6.093      |
| 3029 x Granger | 7.115   | 14.620  | 4.760      |
| 3053 x Hadim  | 7.950   | 11.316  | 6.260      |
| 3048 x Melrose | 7.065   | 10.376  | 5.963      |
| Ultrillo     | 9.265   | 11.970  | 4.730      |
| 3053 x Melrose | 6.588   | 13.476  | 9.760      |
| Sahin x Hadim | 6.707   | 13.404  | 8.647      |
| 3031 x Granger | 7.491   | 9.636   | 4.820      |
| 3053 x Ultrillo | 8.278   | 9.791   | 4.440      |
| 3055 x Melrose | 6.421   | 8.800   | 7.813      |
| 4028 x Hadim  | 7.403   | 9.505   | 6.340      |
| Melrose      | 7.447   | 10.802  | 5.973      |
| 3057 x Granger | 7.015   | 9.015   | 9.007      |
| Sahin x Melrose | 9.396   | 10.462  | 10.063     |
| 4053 x Hadim  | 7.368   | 13.679  | 7.443      |
| 4028 x Ultrillo | 7.250   | 9.291   | 8.887      |
| 3057 x Hadim  | 7.758   | 9.267   | 6.140      |
| Sahin x Ultrillo | 7.339   | 9.839   | 7.700      |
| 3048 x Ultrillo | 6.230   | 8.636   | 6.817      |
| 4028 x Melrose | 6.571   | 9.440   | 7.303      |
| Granger      | 8.195   | 10.846  | 5.297      |
| 3057 x Granger | 7.682   | 8.893   | 5.380      |
| 4028 x Granger | 5.458   | 9.261   | 7.197      |

| Mean         | 7.531 c | 11.052 a | 9.510 b | 9.364 a | 6.789 c | 13.267 a | 10.792 b | 10.283 b | 0.142 c | 0.463 b | 0.510 a | 0.732 a |

**Table 4.** Cold stress applications genotypes peroxidase, superoxide dismutase and proline content and LSD values.
The POX content of the genotypes used in the experiment increased due to cold stress. However, the highest POX content of all genotypes was obtained at -8°C cold stress (Table 4).

Scebba et al. (1998); Atıcı and Nalbantoğlu (1999); Lee and Lee (2000); Ozturk and Demir (2002) and Nayyar et al. (2005) reported that plants use enzymatic antioxidant defense mechanisms such as POX to minimize the effects of cold stress. Many previous studies reported that POX is affected by cold stress (Scebba et al., 1998; Atıcı and Nalbantoğlu, 1999; Scebba et al., 1999; Lee and Lee, 2000; Ozturk and Demir, 2002; Nayyar et al., 2005). In these studies, it was determined that the POX contents of cold-resistant plants increased as cold stress increased, whereas in non-cold-resistant plants, a lower POX content was found. Our results are in harmony with the results of these researchers.

Examination of superoxide dismutase content

While the highest average SOD content of all genotypes was obtained with 13.267 units of mg⁻¹ protein in the -8°C cold stress group, the lowest SOD content was obtained from the control group, with 6.789 mg⁻¹. According to the computed Lsdf test -8°C in cold stress to the first group (a), -12°C in cold stress was in the second group (b) and control was in the last group (d) (Table 4).

The highest SOD content as the average of the stress groups in the study was 15,567 units mg⁻¹ protein in the 3053 x Melrose genotype, and the lowest SOD content was determined in the 3053 x Ultrillo genotype, with 6,714 units mg⁻¹ protein. The SOD values of other genotypes were among these values. Among the genotypes used in this study, the 3053 x Melrose, Sahin x Hadim, 4053 x Melrose, Sahin x Melrose and 3029 x Melrose genotypes were take part in first place.

The SOD content of the plants was the highest at -12°C cold stress application. The increase in SOD activity plays a very important role in terms of self-preservation against oxidative stress due to abiotic stress and support of plants in order to maintain their vital functions under stress conditions (Duman et al., 2016). Several studies have shown that various plants exposed to cold stress increase the SOD content in the for plant structure together with stress (Scebba et al., 1998; Atıcı and Nalbantoğlu, 1999; Lee and Lee, 2000; Ozturk and Demir, 2002; Nayyar et al., 2005). They observed an increase in the activity of the SOD and GR enzymes as a result of winter rye plants being exposed to a temperature of 4°C (Keles and Oncel, 2002). An increase in the activity of SOD in stress-exposed plants has been observed (Asada, 1992). In studies conducted on different plants, they observed that the plants exposed to cold stress reacted similarly to those in a study on antioxidant enzymes. Significant increases in the SOD activities of chickpea plants were observed under cold stress (Turan and Ekmecki, 2011; Gensel et al., 2013). Our results were in close agreement with those of previous authors.

Examination of proline content

According to the averages of the genotypes, the highest proline content was obtained in 0.510 1 mol g FW⁻¹ -12°C cold stress group, and the lowest proline content was obtained from the control group, with 0.142 1 mol g FW⁻¹. According to the computed Lsdf test, -12°C in cold stress to the first group (a), -8°C in cold stress was in the second group (b) and control was in the last group (d) (Table 4).

In this study, the highest proline content of the stress groups was determined as 1.365 36 1 mol g FW⁻¹ in the 3031 x Granger genotype, and the lowest proline content was determined as 0.10 1 mol g FW⁻¹ in the 4009 x Melrose genotype. 3031 x Granger, 3055 x Melrose, Ultrillo, 3057 x Hadim and Sahin x Hadim genotypes were take part in the first place. In general, genotypes (except for 3031 x Granger) were obtained in the application of cold stresses with the highest proline content -12°C.

One of the first reactions in plants against cold stress is the accumulation of a large quantity of different osmotic preservatives. Proline, a common osmolyte found in plants, accumulates particularly when plants respond to cold stresses (Scebba et al., 1998; Atıcı and Nalbantoğlu, 1999; Lee and Lee, 2000; Ozturk and Demir, 2002; Nayyar et al., 2005). In this study, the proline content of pea genotypes increased with increasing cold stress Ozturk and Demir (2002); Nayyar et al. (2005). Proline causes the retention of cellular water by controlling turgor and also leads to the formation of a sheath of water around membranes and macromolecules. This occurs to preserve these structures and remove free radicals. When the results of our study were examined, it was observed that the 3031 x Granger and 3055 x Melrose genotypes had high proline values.

In this research, we attempted to reveal the effects of cold stress on plant growth in 28 pea genotypes in fully controlled plant breeding greenhouses and laboratories in 2015 and the relationship between biochemical or physical defense mechanisms produced by plants against cold stress.

This study was conducted to determine the reactions of the following varieties to cold stress:

1. With respect to cold damage, the Melrose, Falcon, Granger, 4053 x
Melrose, 4053 x Hadim, Hawk x Hadim and 3057 x Melrose genotypes were examined.

2. When POX content was examined, the 3057 x Melrose, 4053 x Melrose, 3029 x Melrose, 3029 x Granger and 4053 x Hadim genotypes displayed the highest values.

3. When SOD content was examined, the 3053 x Melrose, Sahin x Hadim, 4053 x Melrose, Sahin x Melrose and 3029 x Melrose genotypes displayed the highest values.

4. When proline content was examined, the 3031 x Granger, 3055 x Melrose, Ultrillo, 3057 x Hadim and Sahin x Hadim genotypes displayed the highest values.

As a result, when the effect of cold stress on the enzyme activities in the leaves of pea plants is considered, among the varieties, the 3031 x Granger and 3055 x Melrose varieties were determined as the most resistant. It was determined that cold stress significantly changed the antioxidant enzyme activities of all genotypes, and it was observed that genotypes vary greatly with regard to antioxidant defense systems developed against cold stress. In future research, along with the genotypes featured in this study, all pea genotypes should be examined with regard to cold stress tolerance, and resistant genotypes should be identified and their inclusion in breeding programs encouraged.

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