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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 superoxidase 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 Faktörlü Faktöriyel Deneme" desenine göre üç tekerrürlü olarak kurulmuştur ve Selçuk Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü tam kontrollü araştırma serası ve laboratuvarlarında 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 konulmaya çalışılmıştır. Araştırma sonuçlarına göre; soğuk zararı bakımından Melrose, Şahin, Granger, 4053 x Melrose, 4053 x Hadim, Şahin x Hadim ve 3057 x Melrose, peroksidaz içeriği incelendiğinde 3057 x Melrose, 4053 x Melrose, 3029 x Melrose, 3029 x Granger ve 4053 x Hadim, süperoksit 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 yapraklarındaki enzim aktiviteleri üzerine etkileri ele alındığında; genotipler içerisinde en dayanıklı olarak 3031 x Granger ve 3055 x Melrose genotipleri ön plana çıkan bu genotipler daha sonra yapılacak olan soğuğa dayanıklılık ıslah çalışmalarında kullanılabilir.

Anahtar kelimeler: POX, Prolin, Soğuğa 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 (Lichtenhaler, 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; Saltweit, 2001). For this, pea plants developed physiological and biochemical strategies against extreme environmental conditions (Nılsen 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; Bancı, 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_2O_2 that arise as a result of oxidative stress (Scebba et al., 1998). Previous authors found that plants increased the amount of SOD, POX and proline in their structures when exposed to cold stress (Scebba et al., 1998; Atıcı and Nalbantoğlu, 1999; Lee and Lee, 2000; Ozturk and Demır, 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 (Fıebelkorn, 2013). Control and cold stress made application SOD, POX separately for the preparation of enzyme analysis extracts was weigh 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_2O_2 and 0.5 ml of enzyme extract. To this prepared solution was added 1 ml of 2.5 M H_2SO_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; Gokmen 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, 2×10^{-5} M KCN and 5.6×10^{-5} 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 Factorıal 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 İnt.	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%1: 0.9115; SU x G int. lsd%1: 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., 1983 a; Auld et al., 1983 b; Eteve, 1985; Bourıon 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 -8⁰C 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., 1983 a; Auld et al., 1983 b; Eteve, 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	Peroxıdase (POX)	Superoxıde dısmutase (SOD)	Prolıne
Stress application (SU)	2	261.716[**]	897.736[**]	3.362[**]
Genotypes (G)	27	22.198[**]	44.345[**]	0.619[**]
SU x G İnt.	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 (Scebba 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.

Table 4. Cold stress applications genotypes peroxidase, superoxide dismutase and proline content and LSD values.

Genotype	POX				SOD				Prolin			
	Control	-8⁰C	-12⁰C	Mean	Control	-8⁰C	-12⁰C	Mean	Control	-8⁰C	-12⁰C	Mean
Sahin	6.651	9.207	8.819	8.226 ijk	7.533	12.040	11.560	10.378 j	0.030	0.436	0.648	0.371 g
4009 x Melrose	6.817	8.819	8.238	7.958 ckl	7.240	10.793	10.167	9.400 l	0.016	0.136	0.151	0.101 pq
Bolero x Melrose	9.219	11.303	11.029	10.517 d	5.250	11.313	10.200	8.921 n	0.047	0.105	0.129	0.094 q
3057 x Melrose	9.037	14.743	12.830	12.203 a	6.057	14.537	12.443	11.012 h	0.074	0.120	0.178	0.124 opq
4053 x Melrose	9.753	16.782	10.490	12.341 a	7.167	19.000	14.597	13.588 c	0.026	0.168	0.256	0.150 n-q
3029 x Melrose	7.388	16.270	13.470	12.376 a	6.093	16.890	15.623	12.869 e	0.203	0.569	0.645	0.472 f
3029 x Granger	7.115	14.620	12.919	11.551 b	4.760	17.760	13.410	11.977 f	0.058	0.271	0.289	0.206 klm
3053 x Hadim	7.950	11.316	9.327	9.531 ef	6.260	11.660	9.743	9.221 lm	0.056	0.329	0.272	0.219 kl
3048 x Melrose	7.065	10.376	8.554	8.665 hö	5.963	9.583	8.957	8.168 pq	0.427	0.457	0.268	0.384 g
Ultrillo	9.265	11.970	10.951	10.729 cd	4.730	10.437	8.717	7.961 q	0.053	0.934	1.074	0.687 c
3053 x Melrose	6.588	13.476	11.950	10.671 cd	9.760	20.173	16.767	15.567 a	0.449	0.581	0.200	0.410 g
Sahin x Hadim	6.707	13.404	11.443	10.518 d	8.647	20.570	15.337	14.851 b	0.227	0.641	0.760	0.543 e
3031 x Granger	7.491	9.636	8.127	8.418 ij	4.820	10.170	9.003	7.998 pq	0.139	2.072	1.883	1.365 a
3053 x Ultrillo	8.278	9.791	8.746	8.938 gh	4.440	8.890	6.813	6.714 s	0.164	0.265	0.470	0.300 ij
3055 x Melrose	6.421	8.800	7.408	7.543 lm	7.813	15.227	11.023	11.354 g	0.233	0.888	1.142	0.754 b
4028 x Hadim	7.403	9.505	8.388	8.432 i	6.340	10.743	8.430	8.504 o	0.099	0.231	0.264	0.198 k-n
Melrose	7.447	10.802	9.403	9.217 fg	5.973	14.590	11.570	10.711 i	0.161	0.307	0.355	0.274 ij
3057 x Granger	7.015	9.015	7.640	7.890 kl	9.007	13.757	9.920	10.894 hi	0.087	0.483	0.510	0.360 gh
Sahin x Melrose	9.396	10.462	9.812	9.890 e	10.063	16.790	12.637	13.163 d	0.074	0.616	0.708	0.466 t
4053 x Hadim	7.368	13.679	11.956	11.001 c	7.443	12.610	9.743	9.932 k	0.134	0.192	0.202	0.176 l-o
3029 x Ultrillo	7.250	9.291	8.788	8.443 i	8.887	13.567	10.887	11.113 gh	0.134	0.190	0.202	0.175 l-o
3057 x Hadim	7.758	9.267	8.548	8.524 hi	6.140	11.100	9.270	8.837 n	0.127	0.765	0.977	0.623 d
Sahin x Ultrillo	7.339	9.839	8.288	8.489 hi	7.700	13.750	9.123	10.191 j	0.126	0.197	0.156	0.159 mn
3048 x Ultrillo	6.230	8.636	7.082	7.316 mn	6.817	10.883	8.803	8.834 n	0.141	0.196	0.398	0.245 jk
4028 x Melrose	6.571	9.440	7.720	7.910 kl	5.297	9.647	7.737	7.560 r	0.122	0.484	0.575	0.394 g
Granger	8.195	10.846	9.839	9.627 ef	7.303	13.590	11.780	10.891 hi	0.343	0.513	0.610	0.488 f
3057 x Granger	7.682	8.893	8.116	8.230 ijk	5.380	10.523	8.777	8.227 p	0.121	0.385	0.417	0.308 hi
4028 x Granger	5.458	9.261	6.402	7.040 n	7.197	10.883	9.143	9.074 mn	0.116	0.429	0.546	0.364 g
Mean	7.531 c	11.052 a	9.510 b	9.364	6.789 c	13.267 a	10,792 b	10.283	0.142 c	0.463 b	0.510 a	0.732

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 Demır (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 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 (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 Ekmekcı, 2011; Genısel 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 Lsd 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, Utrillo, 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 prolıne content -12°C.

One of the first reactions in plants against cold stress is the accumulation of a large quantity of different osmotic preservatives. Prolıne, 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 Demır, 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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