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DETERMINATION OF PHYSIOLOGICAL AND BIOCHEMICAL REACTIONS OF DIFFERENT PEA VARIETIES AND LINES UNDER CHILLING STRESS

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ABSTRACT

Under field conditions, growth parametres like fresh weight and leaf number, superoxide dismutase (SOD), catalase (CAT), ascorbic peroxidase (APX) enzymes in the antioxidant defence sytems are analysed on 12 lines and two cultivars of pea seedlings which are specified as tolerant against cold. Besides the relationship between the protection mechanisms from abiotic stress conditions (change on the antioxidant enzymes) on the pea plant is tried to be revealed. In terms of macro and microelement accumulation, considering control plants, the effect of low temperature application is not observed in Mn, Mg, Cu microelements. However, approximately ten times decrease is observed on Fe and Zn accumulations. It is also observed that there is not a change on K and Ca accumulation of the plants considering control plants. It is seen that with the low temperature application, there are significant increases on CAT, APX and SOD enzyme activities which are among the antioxidant enzymes of the plants.

Keywords: pea, enzyme activity, CAT, APX, SOD, chilling stress.

AIMS AND BACKGROUND

Pea is a cool and temperate climate plant. It is grown nearly in all regions of the temperate zone while its broadest cultivation area is in the Asian continent and the most productionand the yield rate is in the European continent. Its cultivation is mostly done in developed countries. Plants are affected significantly when they are exposed to one of the various abiotic and biotic stress factors. The exposure of the plants to these stress factors prevents them to show their genetic characters and reach

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the maximum yield potentials. Abiotic stresses which are main reason of yield loss in the world, decrease the average yield rate of the significant products about 50% or more[1]. Cold is one of the abiotic stresses which restrains the yield quality and the yield rate of plants[2]. However some plants, when they are exposed to low temperatures for a length of time, depending upon their genetic structures, they have the ability to increase tolerance against cold. It is known that this event which is identified as cold harmony decreases the damage caused by cold[3]. Therefore, revealing the physiological and biochemical mechanisms which ensure the chilling tolerance and adaptation on plants is significantly important on increasing the productivity and developing plants which are tolerant to chilling. In genetic variations, plants have different reactions like setting seed, shooting against chilling and as in the progress activities in terms of physiological events like plasma membrane function and photosynthesis. Thus, it became compulsory to explore and develop tolerant strains against stress factors like chilling, even in sensitive level within genetic source. Mock and Eberhart[4] from corn germplasm, Patterson and Payne[5] from tomato germplasm; explored tolerant strains against chilling. It is known to cause adverse structural and biochemical changes such as protein denaturationin plants, enzyme inactivation, disrupting the membrane structure, motion of water, ions and organic solvents, respiratory substrate amounts and especially photochemical reduction of activity in the chloroplasts of stress conditions[6]. When plants are exposed to these stress conditions for a long period of time, depending upon CO_2 assimilation limitation, their electron transport reactions also became inactive[7]. Excessive reductions in photosynthetic election systems cause the formation of active oxygen types (AOT) like super oxide radical (O_2^-), singlet oxygen ($O^1{}_2$), hydrogen peroxide (H_2O_2) and hydroxil radical (HO) (Refs 8–10). Active oxygen types are highly reactive and they cause the formation of oxidative reactions like lipid peroxidation, protein denaturation, DNA mutations, pigment fragmentation and enzyme inactivation on plants[11–15] and they spoil the hemeostatics of organisms[16]. Plants have interior defence systems which catch and remove toxic products to protect themselves from the damages of AOT. These mechanisms contain antioxidants like ascorbate, glutathione and tocopherol with antioxidant enzyme systems like superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), glutathione reductase (GR) and ascorbate peroxidase (APX) and thus, destruction reactions of active oxygen types are kept minimum[14,17]. The relationship between the activity of antioxidant systems and stress durabilities of plants is revealed in various stress conditions (drought, cold, high temperature, air pollution, radiation, herbicide, etc.[17–28]

In this study, under field conditions growth parametres like fresh weight and leaf number; superoxide dismutase (SOD), catalase (CAT), ascorbic peroxidase (APX) enzymes in the antioxidant defence sytems are analysed on 12 lines and two cultivars of pea seedlings which are specified as tolerant against cold. Besides the relationship between the protection mechanisms from abiotic stress conditions (change on the antioxidant enzymes) on the pea plant is tried to be revealed by doing macro- and microanalysis.

EXPERIMENTAL

Under Van ecological conditions by winter sowing, 12 pea lines which overcame winter (101917, 1121918, 10431, B6, 110121, 1101545, 1084222, 1131522, B8, 1131556, 1103220 and 110121-1) and two different cultivars (Winner and Karina) were used. These materials were obtained from cross breeding of peas which are from white-flowered pea population and turned into lines and wild peas which were collected from the wild. Crossbresd members were turned into lines by using the tandem selection method. At the present time lines are from F8 generation. 'B' codded members are parents. The main criterion for selection of the lines was their winter tolerance (Table 1).

Abiotic stress resistances. After pea seedlings (*Pisum sativum* ssp arvense) belonging to the pea cultivars and lines which are used in the experiment are germinated, they are placed in vermiculate to apply chilling stress. Then, pea seedlings are waited in the climate room, 27/16°C temperature compensated and under 1600 μmol $m^{-2} s^{-1}$ lux with 14/10 h light/darkness periods at 65–70% damp for 10 days long after doing diversion as three repetitive two paralels and one each plant from both genotypes to sand filled plastic glasses in the form of control and practice. After 4–5 real leaves formed on plants, the temperature is decreased to 9/4°C (light/dark) and lux is decreased to 100 μmol $m^{-2} s^{-1}$ and they are waited for 6 days long[29].

Fresh weight measurements. Green parts fresh weights which are taken from each renewal are determined by weighing on precision scale. Antioxidant enzyme activities: As a result of cold stress implementation, antioxidant enzyme [(superoxide dismutase (SOD), catalase (CAT), ascorbic peroxidase (APX)] activities are specified by using spectrophotometric method. Superoxide dismutase (SOD) activity, in accordance with Cakmak and Marschner[30], is measured according to the method of degrading the NBT (nitro blue tetrazolium chloride) by O_2^- under light. Ascorbic peroxidase (APX) activity is done in accordance with Cakmak and Marschner[30], by measuring the oxidation of ascorbate at 290 nm (E = 2.8 mM cm^{-1}). Catalase activity (CAT) is measured by basing on the decay rate of H_2O_2 at 240 nm (E = 39.4 mM cm^{-1}) (Ref. 30). K, Ca, Fe, Zn, Cu, Mn and Mg contents are rmeasured by using the Atomic Absorbtion device (Thermo Fisher Scientific iCE 3300) in accordance with Kacar[31].

Table 1. Using lines numbers in the trials

No	Line No	No	Line No
1	1103220	8	1121918
2	1084222	9	1131556
3	B-6	10	B-8
4	110121	11	101917
5	1101545	12	10431
6	110121-1	13	Carina
7	11131522	14	Winner

RESULTS AND DISCUSSION

Fresh weights of plants belonging to the pea lines decreased compaired to control plants with the low temperature application. The highest value was obtained from 2, 10, 12 lines and on the other hand the lowest value was obtained from 13, 8 and 14 (Table 2). There has become a decrease on the leaf numbers of the plants as against control with the low temperature application. The cultivars with the lowest leaf number has become number 13 and 14. In terms of leaf number, other lines are involved in the same statistical group (Table 2). With the low temperature application on pea plants, to determine the reaction of plants against low temperature application and to state if there is a relationship between development and antioxidative defence system, CAT, APX and SOD enzymes which are among enzymatic antioxidants are observed. With the low temperature application the CAT activity of plants increased quite a lot as against control, while a difference between lines and cultivars is not observed during the control application; generally during the low temperature application a difference is not found out between the lines as well. The highest value has been found at the line number 2 and the lowest value is at the line number 8. In terms of APX activity, both in point of control and application although overall of cultivars and lines are among the same statistical group, number 2 genotype has been found at the highest value and number 14 cultivar has been found at the lowest value. According to the control application, APX activity on the plants which are exposed to low temperature application has increased to a large extent (Table 2). Similarly, SOD activity has also showed increase compaired to control plants and again the highest value has been at line number 2, the lowest value has been found at cultivar 14. The overall of other lines has been found in the same range (Table 2). Pea lines and cultivars when they are analysed in terms of Ca and K accumulations, with the low temperature application although some slight decreases have been seen on K accumulations there has not been any switch on Ca accumulation. In terms of K accumulation the overall of the lines has been seen in the same statistical range, the highest K accumulation has been seen on the line number 1 and the lowest has been seen on the line number 12. As for Ca accumulations, with the low temperature application all lines have been seen in the same range; while some of the lines are increasing, decreases have been in some of them as against control (Table 3). When microelement accumulations on the leaves of pea plants are analysed, in terms of Cu accumulation a change has not been seen typically neither among lines nor between applications. However, with the low temperature application Fe accumulations have been reduced about ten fold compaired to control plants. In Fe accumulation a difference has not been seen among the lines and cultivars as well as Cu accumulation (Table 3). Unlike iron, increase has been seen in Zn accumulations on the leaves of pea plants which are applied at low temperature, as against control plants. In both application a difference could not be found among lines and cultivars in terms of Zn accumulation (Table 3). In respect to micro- and macroelement accumulation the effect of low temperature application

Table 2. Fresh weight of green part (FGGP) (g), leaf numbers (LF) (number), CAT, APX (μmol/min/mg FW) and SOD (U/g FW) enzyme activity of control and low temparature applied plants

Geno-types	FGGP		LF		CAT		APX		SOD	
	cont.	appl.	cont.	appl.	cont.	appl.	cont.	appl.	cont.	appl.
1	1.19 adA	0.60ceB	11.51 aA	5.10aB	0.31aB	3.18abA	0.62acB	3.38abA	168dB	621.33abA
2	1.51 aA	1.03aB	10.20 abA	5.10aB	0.48aB	4.46aA	0.74abB	3.67aA	173cdB	701.67aA
3	1.28 adA	0.64ceB	10.67 abA	5.03aB	0.38aB	3.96abA	0.79aB	3.38ab A	335aB	432.00ceA
4	1.05 bdA	0.66ceB	11.63 aA	5.2aB	0.26aB	1.72bA	0.52acB	3.05abA	85eB	413.33ceA
5	1.22 adA	0.61ceB	9.60 adA	4.3bcB	0.34aB	3.07abA	0.68abB	4.00aA	85.6eB	357.67deA
6	1.15 adA	0.68ceB	11.60 aA	5.1aB	0.27aB	3.11abA	0.64acB	3.62abA	119deB	341.00deA
7	1.06 bdA	0.70cd	6.75 eA	4.76abB	0.37aB	2.06abA	0.29ceB	3.00abA	242.6bcB	383.67deA
8	1.12 adA	0.50deB	9.33 acA	4.76abB	0.61aB	1.42bA	0.18deB	4.19aA	186.6cdB	488.00bdA
9	1.17 adA	0.59ceB	8.70 beA	4.63abB	0.22aB	1.93abA	0.37ceB	4.53aA	181.0cdB	378.67ceA
10	1.42 abA	0.95abB	7.53 ceA	4.76abB	0.15aB	2.03abA	0.30ceB	3.57aA	120deB	468.00bdA
11	0.98 ceA	0.56ceB	10.93 abA	4.20abB	0.16aB	2.40abA	0.09eB	3.38abA	258.obB	389.67ceA
12	1.37 acA	0.79bcB	9.63 adA	4.76abB	0.16aB	4.46aA	0.19deB	3.29abA	287.3bB	575.67acA
13	0.65 eA	0.44eB	7.17 deA	3.80 cB	0.22aB	2.17abA	0.21deB	1.90bcA	154.0deB	240.33eA
14	0.92 deA	0.54deB	6.93 eA	3.83 cB	0.21aB	2.23abA	0.12deB	1.43cA	260.0bB	236.00eA

Differences between means with the same small letter in the same column are not significant at $P \leq 0.05$. Differences between means with the same big letter in the same row are not significant at $P \leq 0.05$.

Table 3. K, Ca, Fe and Zn accumulation of control and low temperature applied plants

Geno-types	K		Ca		Fe		Zn	
	cont.	appl.	cont.	appl.	cont.	appl.	cont.	appl.
1	0.12A	0.169aA	0.045 acA	0.036 B	0.014A	0.0020 B	0.005B	0.012A
2	0.13A	0.100cd B	0.030c B	0.033A	0.011A	0.0013 B	0.004 B	0.009A
3	0.13A	0.120acA	0.027c B	0.034A	0.010A	0.0016 B	0.005 B	0.010A
4	0.12A	0.120acA	0.040ac B	0.047A	0.012A	0.0016 B	0.004 B	0.014A
5	0.12A	0.104bcA	0.042acA	0.027 B	0.011A	0.0013 B	0.005 B	0.011A
6	0.13A	0.126acA	0.033bcA	0.027 B	0.012A	0.0016 B	0.004 B	0.011A
7	0.12A	0.102bcA	0.038acA	0.031 B	0.010A	0.0006 B	0.005 B	0.008A
8	0.12A	0.149abA	0.038acA	0.033 B	0.011A	0.0006 B	0.005A	0.005A
9	0.13A	0.120acA	0.031cA	0.032A	0.010A	0.0013 B	0.004 B	0.013A
10	0.12A	0.112acA	0.034bcA	0.034A	0.011A	0.0006 B	0.004 B	0.012A
11	0.12A	0.110ac A	0.058aA	0.035 B	0.009A	0.0006 B	0.004 B	0.012A
12	0.13A	0.091bc B	0.026c B	0.039A	0.011A	0.0010 B	0.004 B	0.023A
13	0.12A	0.074c B	0.045acA	0.044A	0.011A	0.0020 B	0.005 B	0.021A
14	0.13A	0.098cd B	0.055abA	0.045 B	0.010A	0.0010 B	0.008 B	0.015A

Differences between means with the same little letter in the same column are not significant at $P \leq 0.05$.
Differences between means with the same big letter in the same row are not significant at $P \leq 0.05$.

Table 4. Mn, Mg and Cu accumulation of control and low temparature applied plants

Geno-types	Mn		Mg		Cu	
	cont.	appl.	cont.	appl.	cont.	appl.
1	0.014 bA	0.006aB	0.002 aA	0.0006 bB	0.036aA	0.034abA
2	0.010 bA	0.006aB	0.001 aA	0.0013 bB	0.036aA	0.029ab B
3	0.011bA	0.009aB	0.003 aA	0.0013 bB	0.035aA	0.030ab A
4	0.012 bA	0.004aB	0.002 aA	0.0016 bB	0.034aA	0.031ab A
5	0.015 bA	0.007aB	0.001 aA	0.0013 bB	0.033aA	0.033abA
6	0.014bA	0.006aB	0.002 aA	0.0016 bB	0.035aA	0.034abA
7	0.013 bA	0.008aB	0.002 aA	0.0006 bB	0.036aA	0.030ab A
8	0.010 bA	0.008aB	0.003 aA	0.0006 aB	0.031a B	0.038aB
9	0.013bA	0.006aB	0.002 aA	0.0013 bB	0.033aA	0.031ab A
10	0.012bA	0.005aB	0.001 aA	0.0006 bB	0.035aA	0.031ab A
11	0.018abA	0.007aB	0.002 aA	0.0010 bB	0.029aA	0.021b B
12	0.013 bA	0.009aB	0.001 aA	0.0020 bB	0.032aA	0.028ab A
13	0.017abA	0.006aB	0.003 aA	0.0016 aB	0.036a A	0.040aA
14	0.027aA	0.008aB	0.002 aA	0.0010 aB	0.028a B	0.039aA

Differences between means with the same little letter in the same column are not significant at $P \leq 0.05$.
Differences between means with the same big letter in the same row are not significant at $P \leq 0.05$.

has not been seen in Mn, Mg and Cu microelements as against control plants but approximately tenfold decrease has been observed in Fe and Zn accumulations (Table 4). The K and Ca accumulations of plants have also been observed not to change as against control plants. With the low temperature application, critical increases have been observed in CAT, APX and SOD enzyme activities which are from antioxidant enzymes of plants. In general, while there are differences among lines in terms of growing parametres and enzyme activities, a difference has not been observed in respect to macro and microelement among others except K.

In the seedling period, under field conditions, by using selection breeding method and by practising chilling stress with biochemical and physiological methods to pea, lines are specified as tolerant against chilling and to two pea cultivars which are among cool and temperate climate plants and whose cultivation has been done in various regions of our country, tolerance status of chosen lines has been observed. In other respects, the relationship between the protection mechanisms from cold stress conditions (change on the antioxidant enzymes) is tried to be revealed. Low temperature regime is used in the research of cold effectiveness on pea. For instance Foolad and Lin[32], in their study on tomato, they stated the cold tolerance index with the green parts weight and dry matter production by using 10/15°C light/dark temperature in the vegetative period. Furthermore, in this study it is shown that chilling tolerance in tomato can be hereditary and genetic transitive. In our study although all pea lines and cultivars are winter types, with cold application it is seen that there are decreases in their growing yet under low temperature, in general lines has showed the same performance.

Protection from the harms of stress can be a result of the existance of antioxidative enzyme systems which include highly SOD, CAT, GR and APX activities which are increased with genetics and stress as in the studies that are worked on some plant species before[33]. The presence of SOD in plants is very importatant and its quantity is a measure of the antioxidandt properties of plants[34]. SOD enzyme activation which abolish superoxide radical that is among active oxygen derivatives, owned different SOD activities in pea lines and cultivars which are applied chilling stress. Except number 14 cultivar, in all other cultivars and lines, SOD activities increased as against control under chilling stress. SOD activities of lines has been found higher than cultivars. The importance of operating the enzyme systems in terms of chilling tolerance, explicitly confronted us. Plants produced superoxide radical under chilling stress and lines whose capacity to annihilate this produced radical is higher, displayed a better strength against chilling. The increase of SOD activity with cold application is also stated by other researchers[35,36]. These findings indicate that SOD enzyme activity has a place in pea in terms of chilling tolerance characteristic. Superoxide dismutase enzyme annihilates the superoxide radical yet hereat another matter whose toxic characteristic is very high, hydrogen peroxide has been formed. Enzymes that are effective for fragmentation of hydrogen peroxide (detoxification) are CAT and APX. CAT enzyme activity seriously proceed high in all cultivars and lines on pea plants which are applied

chilling stress as against control plants. There have been differences among cultivars and lines as well. While the highest CAT activity has been found at line numbers 2 and 12, the lowest CAT activity has been found at line numbers 4 and 8. In terms of CAT activity a difference is not found out between lines and cultivars in general. In this case, an opinion has been built on capable genotypes whose tolerances are also high in respect of increasing the CAT enzyme activity. Likewise, increase has been observed in APX activity as against control plants. While APX enzyme activities of all lines have been found high, in cultivars they have been found in the same range and lower. It shows that lines which are used in the experiment are more durable than 13th and 14th cultivars which are winter types. The relationship between the activity of antioxidant systems and stress durabilities of plants is revealed in various stress conditions (drought, cold, high temperature, air pollution, radiation, herbicide, etc.)[18–26,28,37]. Stress affects mineral element uptake, growing of stems, food flow in soil and nutrient uptake on plants. The nutrient uptake of plant which is exposed to a stress has an important role on the tolerance of that plant against stress. Genotypes which are developed by selection and can be adapted to the stress conditions give an outstanding performance in yield. Compared with control plants, decreases are observed on the Fe uptake of plants which are applied drought. In the same way, it is stated that there is a significant increase on the Fe, Cu, Zn uptake of Menemen cultivar which is grown under 7-day short-time drought application and this cultivar is a drought-tolerant cultivar. In terms of element uptake, as drought stress and chilling stress mechanisms resemble, similar results came out in the study which we did by applying chilling stress on pea. K and Mn accumulations on leaf organs of pea cultivars and lines which are applied and unapplied chilling stress are examined and as against control plants, decreases in both two ions are observed on plants which are applied chilling stress. While the highest K accumulation has been seen on the lines, the lowest K accumulation has been seen on cultivars. As for Mn accumulation, while there are decreases as against control, no difference was found among genotypes. Serious decreases are observed about Mn uptake on plants which are grown under long-term drought stress. Culture types whose decrease on nutrient element uptake is less are more tolerant against stress. Besides no mutation is occurred on Mg and Ca accumulations of pea plants which we applied chilling stress in contrast with control plants, no difference was found among genotypes. Serious decreases are observed on total nutrient element uptake and on the concentration of mineral elements on plants growing under drought and salty conditions, chilling with the decrease of water.

CONCLUSIONS

Fresh weight of the plants belonging to the pea lines decreased compared to the control plants with low temperature application. CAT activities of plants showed considerable increase compared to the control plants with low temperature application. APX activitiy of plants that applied low temperatue increased compared to control application. Low

temperature application decreased K accumulation of cultivars and lines. However, there was no change in Ca accumulation. Microelements accumulation in leaves of pea plants when examined, there was no difference neither lines nor application in Cu accumulation. Zn accumulation of leaves of pea plant increased compared to control plants with low temperature application.

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