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ORIGINAL RESEARCH PAPER

Influence of long-term cold stress on enzymatic antioxidative defense system in chickpea (*Cicer arietinum* L.)

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Abstract
Abiotic stresses such as cold, heat, and drought are the main causes of universal crop losses. Plants have generated adaptive responses which prevent them from oxidative damage caused by environmental stresses. The present research aimed to evaluate the effect of cold stress on lipid peroxidation and antioxidant enzyme activity in the leaves of eight cultivars / advanced lines of chickpea (*Cicer arietinum* L.). Three-week-old plantlets were subjected to cold stress (0°C) for 24 or 48 hours. Selected antioxidant enzyme activity and oxidative status of chickpea plantlets under cold stress were determined. In most genotypes, catalase and ascorbate peroxidase activities were increased and guaiacol peroxidase activity decreased under stress conditions but the activity of superoxide dismutase remained almost constant. Based on its ranking, *Cicer arietinum* 'Saral', a newly released cold-resistant Iranian chickpea cultivar, had the strongest, and FLIP 05-77C had the weakest antioxidative defense system under low temperature stress.

Keywords
oxidative stress; chickpea; cold stress; lipid peroxidation

Introduction

Chickpea (*Cicer arietinum* L., Fabaceae), the only cultivated species belonging to the genus *Cicer*, is an agronomically and economically important grain legume currently grown in 56 countries (13.7 Mt total yield from 14 Mha, with a productivity of 1.01 t ha^{-1}) [1]. In 2014, India produced 9.88 Mt of chickpea and was ranked first in the world; Iran was seventh with 260,000 t [1]. Chickpea productivity records in the last four decades reveal a decline in Turkey, Pakistan, and Iran [2]. Due to a rapid increase in world urban population (2 billion) over the last 30 years, increasing the yield of crops to produce human food is more than ever necessary [3]. Chickpea cultivation is a vital part of agriculture in the developing countries and the crop plays an important role in mankind's nutritional system as a major source of high quality dietary protein and carbohydrate, and an appropriate alternative to animal sources of protein. This crop also restores and maintains soil fertility by nitrogen fixation and is very suitable to fit in to various cropping systems [4,5].

Ambient temperature determines the geographical distribution of crops and has a direct effect on their growth. It has been demonstrated that plants can tolerate temperatures slightly higher or lower than optimal for their growth. It is believed that among the abiotic stresses, heat and cold are the most common causes of stress in chickpea production in the moderate and cold Mediterranean regions [5]. Survival of plants is

noticeably reliant on their ability to adapt to environmental conditions. Because of their immovability, plant survival depends on responding sensitively to environmental stimuli by adjusting or adapting physiologically. Lacking suitable signal perception and responding rapidly can result in damage and potentially death [6].

Cold is a major environmental stress which imposes extensive formation of reactive oxygen species (ROS) in plants. These are partially-reduced forms of atmospheric oxygen and developed from disrupted processes in the electron transport chains. Overproduction of ROS brings about destruction of macromolecules, DNA, proteins, lipids, and carbohydrates [7]. The very effective enzymatic and nonenzymatic antioxidant defense mechanisms of plants protect them against oxidative stress damage by scavenging ROS and determine the cascades of uncontrolled oxidation in order to increase plant tolerance to stress [8,9]. Although an excess of ROS is toxic to plant cells, a certain level of ROS production is required for a successful response to stress. ROS play an important signaling role in plants, influencing the expression of some genes and consequently controlling many processes such as abiotic stress responses and pathogen defense [7,10,11].

Chickpea is classified as a chilling-sensitive species, thus every year a sudden drop in temperature in fall, freezing temperatures in winter, and late cold spring events result in globally significant losses of chickpea yield (about a 40% overall reduction) because of terminal water deficit as a major reason for stress [12–14]. The major limiting factor in fall-sown chickpea production is low temperature stress, thus the introduction of cold-tolerant chickpea varieties to cultivate in cold climatic zones of Iran is essential. Spring planting of chickpea because of the susceptibility of the crop to cold stress has contributed to low seed yield. Therefore, sowing earlier or as an fall crop will result in a more sustainable growth season, efficient use of soil moisture, and higher yields [15]. In Iran, important breeding programs for increase in cold resistance in order to create high yielding fall planting chickpea cultivars are now underway and a cold-resistant cultivar 'Saral' has recently been released. Screening the ICARDA's Chickpea International Cold Tolerance Nursery is therefore an important step in cold tolerance breeding programs [16]. Evaluation of antioxidant defense system responses in cultivars and advanced lines of cultivated chickpea is a potent device for assessing the existing genetic diversity.

The nature of cold stress, the wide range of plant reactions, and the interactions between environmental stresses all affect cold tolerance. Providing accurate estimates of cold tolerance is a vital tool for effective selection in plant breeding programs. In general, all abiotic stresses, including cold stress, increase the production of ROS, which damage cell membranes, and antioxidant enzymes play an important role in cold tolerance through ROS inactivation. The present study was carried out in order to evaluate the activity of selected antioxidative enzymes and the level of oxidative stress in a newly released variety and some advanced breeding lines of chickpea exposed to cold stress.

Material and methods

Plant material and growth conditions

Eight Iranian or Turkish chickpea cultivars / advanced lines were selected from 124 advanced lines and cultivars based on field studies on their resistance to cold stress. These were: cultivar 'Saral' (resistant), FLIP 03-6C (semiresistant), ILC533 (susceptible), Aziziye-94 (resistant), FLIP 00-39C (susceptible), FLIP 05-77C (susceptible), FLIP 06-173C (resistant), cultivar 'Arman' (semiresistant). All these were provided by the Dryland Agriculture Research Institute (DARI), Maragheh, East Azerbaijan Province, Iran. Seeds of each were sown in pots containing farm soil at a rate of 10 seeds per pot. Plants were grown for 21 days in a growth chamber under lamps producing white and yellow light with 120 μmol m^{-2} s^{-1} photon flux density, with a photoperiod of 16-h day length, 25°C and night (8 h, 18°C) and 75% relative humidity. Cold stress treatment was commenced after 3 weeks of seedling growth under optimal conditions. A control group of 3-week-old plants was sampled on the twenty-first day (NS; nonstressed) and

another two groups were stressed by diminishing temperature as explained below. During 48 hours, the temperature was decreased gradually to 0°C at a rate of 5°C / 12 h, and the plants of treatment groups were then continued at this temperature for 24 (MS – moderate stress) or 48 hours (SS – severe stress). Leaves without petioles were harvested immediately after removal of the plants from the cold exposure room. Samples were obtained from the middle leaves from the apex of each branch of seedlings and rapidly steeped in liquid nitrogen. The samples were preserved at −80°C until analyses were performed.

Activity of enzymes, MDA, H_2O_2, and total soluble protein content assay

For superoxide dismutase (SOD), catalase (CAT), and guaiacol peroxidase (GPX) activity, leaf samples (0.5 g) were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA using a prechilled pestle and mortar. Each homogenate was transferred to centrifuge tubes and was centrifuged at 4°C for 15 min at 15,000 *g*. The supernatant was used for the enzyme activity assays [17]. SOD activity was determined according to the method of Gupta et al. [18] by measuring the inhibition of NBT (nitrobluetetrazolium) reduction at 560 nm. One enzyme unit was defined as the amount of enzyme which could cause 50% inhibition of the photochemical reaction. CAT and GPX activities were assayed as described by Aebi [19] and Panda et al. [20], respectively. The method of Yoshimura et al. [21] was used to assay ascorbate peroxidase (APX). Hydrogen peroxide levels were determined according to Sergiev et al. [22], and MDA was determined using the method of Stewart and Bewley [23]. The protein content in samples was determined using Coomassie Brilliant Blue and measuring the absorbance at 595 nm, according to the Bradford method [24]. Bovine serum albumin was used as a standard.

Statistical analysis

The antioxidant enzyme activities, MDA, and H_2O_2 contents of samples were recorded in a factorial experiment based on a completely randomized design with three replications. The cold stress factor was set at three levels and the genotype factor eight, totaling 72 pots. All data were analyzed by analysis of variance (ANOVA) using the SAS ver. 9.1 (SAS Institute, Cary, NC, USA) and treatment means compared using Duncan's multiple range test.

Results

Effect of cold stress on CAT activity

The activity of CAT was significantly affected by genotype, cold stress treatment, and their interaction (Tab. 1). The highest CAT activity was noted in *Cicer arietinum* 'Saral' (Tab. 2) under MS treatment (Tab. 3). In all cultivars / advanced lines, with the exception of ILC533 and FLIP 05-77C, CAT activity was increased with MS treatment compared to nonstressed plants, and the greatest increase was observed in Aziziye-94. In this line, FLIP 00-39C, and 'Arman', CAT activity was increased under SS conditions compared to the MS treatment, and was decreased in the other cultivars/advanced lines (Fig. 1A). The top five CAT activities in the stressed plants belonged to *Cicer arietinum* 'Saral' (MS), FLIP 00-39C (SS), Aziziye-94 (SS), FLIP 06-173C (MS), and Aziziye-94 (MS); except for FLIP 00-39C, the other three genotypes were resistant to cold stress.

Effect of cold stress on GPX activity

A significant effect of genotype, cold stress treatment, and their interaction on GPX activity was also demonstrated (Tab. 1). The highest GPX activity was observed in *Cicer*

Tab. 1 Analysis of variance for the effect of cold stress on antioxidative defense in chickpea cultivar/advanced lines.

Source of variation	*df*	CAT	GPX	SOD	APX	MDA	H_2O_2
Cold stress	2	$1.27E^{-8}$ **	$5.4E^{-6}$ **	$6.1E^{-4}$ **	$1.8E^{-5}$ **	$9.66E^{-8}$ *	$2.01E^{-2}$ **
Genotype	7	$1.07E^{-9}$ **	$6.1E^{-7}$ **	$1.4E^{-4}$ ns	$1.4E^{-5}$ **	$2.94E^{-7}$ **	$9.32E^{-4}$ ns
Cold Stress × Genotype	14	$2.85E^{-9}$ **	$5.7E^{-7}$ **	$1.4E^{-4}$ ns	$1.0E^{-5}$ **	$8.62E^{-8}$ **	$3.18E^{-3}$ **
Error	48	$1.60E^{-10}$	$1.0E^{-7}$	$1.0E^{-4}$	$1.6E^{-6}$	$3.19E^{-8}$	$6.42E^{-4}$

Significant at $p \leq 0.05$ (*) and $p \leq 0.01$ (**); ns – nonsignificant.

Tab. 2 Comparison of means for antioxidative defense components among different chickpea cultivars / advanced lines under cold stress.

	CAT (units/g FW)	GPX (units/g FW)	SOD (units/g FW)	APX (units/g FW)	MDA (nmoles g^{-1} FW)	H_2O_2 (nmoles g^{-1} FW)
'Saral'	$9.2E^{-5}$ a	$1.55E^{-3}$ a	0.0565 ab	$8.90E^{-4}$ b	$9.60E^{-4}$ cd	0.127 ab
FLIP 03-6C	$7.2E^{-5}$ bc	$1.29E^{-3}$ ab	0.0600 a	$1.28E^{-3}$ b	$9.30E^{-4}$ d	0.132 c
ILC533	$6.7E^{-5}$ cd	$7.90E^{-4}$ c	0.0589 a	$2.15E^{-3}$ b	$1.07E^{-3}$ bcd	0.140 c
Aziziye-94	$8.1E^{-5}$ ab	$1.28E^{-3}$ ab	0.0566 ab	$1.25E^{-3}$ b	$1.39E^{-3}$ a	0.126 b
FLIP 00-39C	$8.3E^{-5}$ ab	$1.09E^{-3}$ bc	0.0585 a	$2.28E^{-3}$ b	$1.14E^{-3}$ bc	0.127 c
FLIP 05-77C	$8.3E^{-5}$ ab	$1.17E^{-3}$ b	0.0474 b	$1.18E^{-3}$ b	$1.42E^{-3}$ a	0.131 c
FLIP 06-173C	$8.4E^{-5}$ ab	$1.25E^{-3}$ ab	0.0571 ab	$1.73E^{-3}$ b	$1.06E^{-3}$ bcd	0.134 c
'Arman'	$5.8E^{-5}$ d	$7.90E^{-4}$ c	0.0597 a	$4.92E^{-3}$ a	$1.18E^{-3}$ b	0.151 a

Means with common letters have no significant differences at $p \leq 0.05$.

Tab. 3 Comparison of means for antioxidative defense components of chickpea among different cold stress treatments.

	CAT (units/g FW)	GPX (units/g FW)	SOD (units/g FW)	APX (units/g FW)	MDA (nmoles g^{-1} FW)	H_2O_2 (nmoles g^{-1} FW)
Control	$5.2E^{-5}$ c	$1.66E^{-3}$ a	0.0569 ab	$1.06E^{-3}$ c	$1.08E^{-3}$ b	0.1033 c
Moderate stress	$9.7E^{-5}$ a	$1.07E^{-3}$ b	0.0517 b	$1.99E^{-3}$ b	$1.15E^{-3}$ ab	0.1333 b
Severe stress	$8.4E^{-5}$ b	$7.30E^{-4}$ c	0.0619 a	$2.83E^{-3}$ a	$1.21E^{-3}$ a	0.1613 a

Means with common letters have no significant differences at $p \leq 0.05$.

arietinum 'Saral' (Tab. 2) in the control treatment (Tab. 3). In all cultivars / advanced lines, except for *Cicer arietinum* 'Saral' and FLIP 00-39C, GPX activity was decreased in MS compared to the NS treatment; the magnitude of the reduction was higher in Aziziye-94, FLIP 05-77C, and FLIP 03-6C. In ILC533 and FLIP 06-173C, GPX activity was increased under SS conditions compared to MS, and was decreased in the other cultivars/advanced lines (Fig. 1B). The top five GPX activities under stressed conditions were in *Cicer arietinum* 'Saral' (MS and SS), FLIP 00-39C (MS), FLIP 06-173C (SS), and Aziziye-94 (MS).

Effect of cold stress on SOD activity

There were no significant differences between the eight chickpea cultivars / advanced lines in terms of SOD activity, but the cold treatment significantly affected its activity

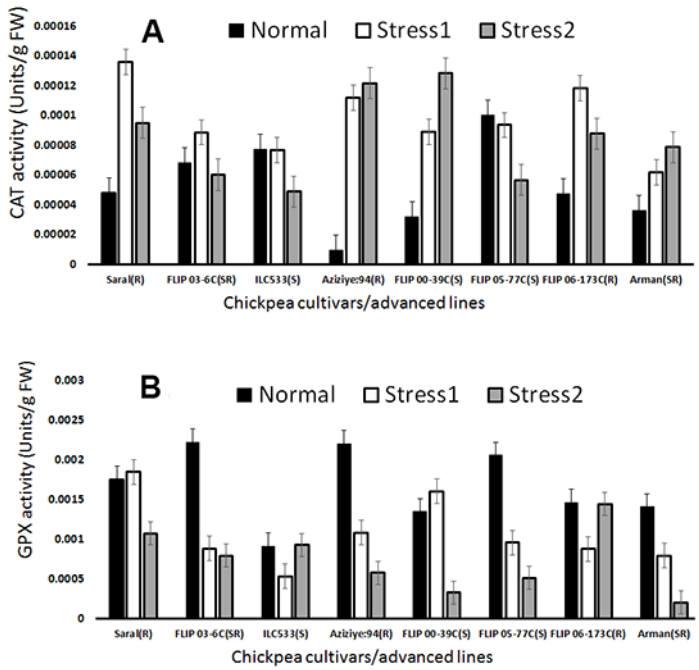

Fig. 1 Effect of cold stress on catalase (**A**) and guaiacol peroxidase (**B**) activity of chickpea. Bars represent standard errors from triplicate experiments. R – resistant; SR – semiresistant; S – susceptible; FW – fresh weight.

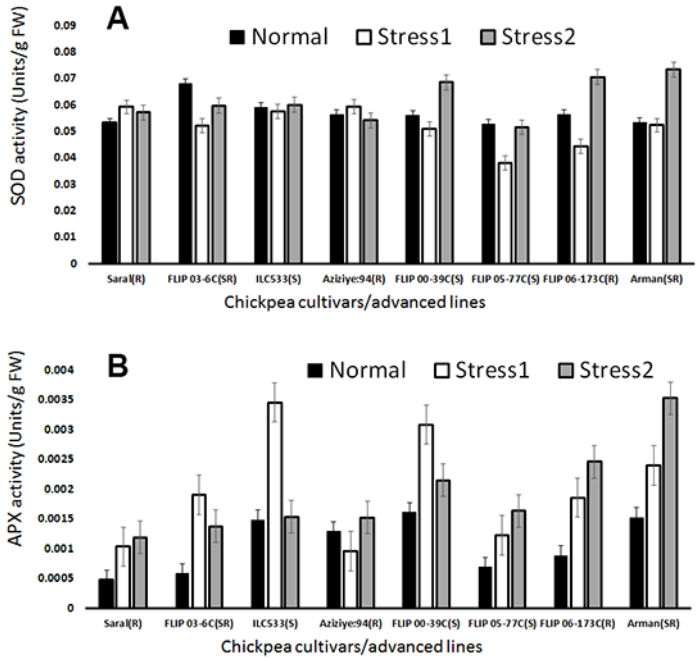

Fig. 2 Effect of cold stress on superoxide dismutase (**A**) and ascorbate peroxidase (**B**) activity of chickpea. Bars represent standard errors from triplicate experiments. R – resistant; SR – semiresistant; S – susceptible; FW – fresh weight.

(Tab. 1). The highest SOD activity was detected under SS treatment, and the lowest was noted in the MS treatment (Fig. 2A, Tab. 3).

Effect of cold stress on APX activity

Significant effects on APX activity of genotype, cold stress treatment, and their interaction were found from the ANOVA (Tab. 1). The highest APX activity was in *Cicer arietinum* 'Arman' (Tab. 2) in the SS treatment (Tab. 3). In all cultivars / advanced lines, except for Aziziye-94, APX activity was increased by the MS treatment compared to that at the optimal temperature. The greatest increase was noted in ILC533, FLIP 00-39C, and FLIP 03-6C. In these three recent advanced lines, APX activity was decreased by SS treatment by comparison to MS, and was increased in the other cultivars / advanced lines (Fig. 2B). The four cultivars exhibiting the greatest APX activities were 'Arman' (SS and MS), ILC533 (MS), FLIP 00-39C (MS), and FLIP 06-173C (MS), indicative of efficient removal of H_2O_2 by these genotypes.

Effect of cold stress on lipid peroxidation

The production of MDA was significantly affected by genotype, cold treatment, and the Cold Treatment × Genotype interaction (Tab. 1). The highest MDA content was in FLIP 05-77C (Tab. 2) under SS treatment (Tab. 3). In *Cicer arietinum* 'Saral', MDA content decreased under cold stress in comparison to other treatments, which is associated with the cold tolerance in this cultivar and indicates its higher membrane stability. The content of MDA in ILC533, FLIP 06-173C, and 'Arman' at various levels of cold stress was nearly constant. In FLIP 03-6C, the MDA content gradually increased in cold-stressed plants comparing to NS plants. Thus, the cold stress caused an increase in the lipid peroxidation of the cell membranes of this line. The MDA content in MS was higher than in other treatments in Aziziye-94, and for SS it was comparable to NS plants. In FLIP 05-77C, the MDA content decreased under MS and increased under SS compared to the NS treatment. In FLIP 00-39C, MDA accumulation in the control plants was lower than in cold-stressed plants and was approximately equal to that in the MS and SS treatments (Fig. 3B). The five lowest MDA contents were found in *Cicer arietinum* 'Saral' (SS and MS), FLIP 03-6C (MS), ILC533 (MS), and FLIP 06-173C (MS).

Effect of cold stress on H_2O_2 content

The ANOVA demonstrated that there were no significant differences between the chickpea cultivars / advanced lines in their H_2O_2 contents, but that the cold treatment

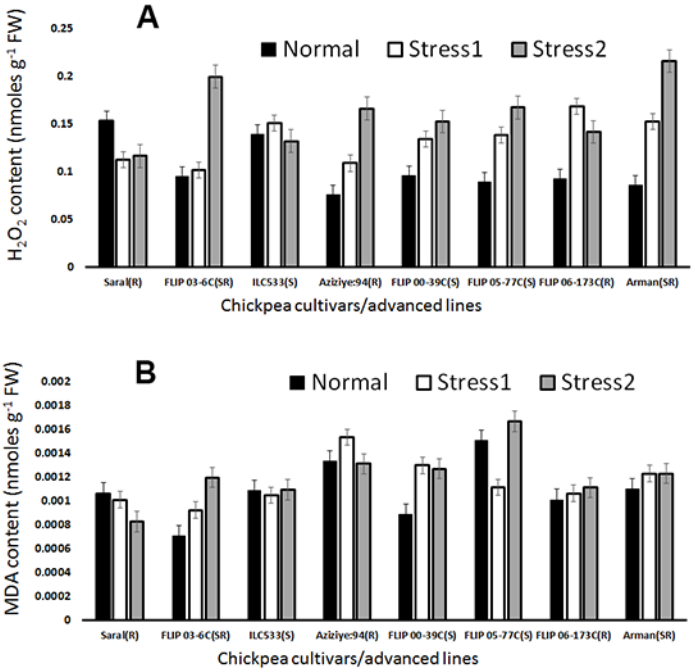

Fig. 3 Effect of cold stress on hydrogen peroxide (**A**) and malondialdehyde (**B**) content in chickpea. Bars represent standard errors from triplicate experiments. R – resistant; SR – semiresistant; S – susceptible; FW – fresh weight.

and Cold Treatment × Genotype interaction in H_2O_2 accumulation were significant (Tab. 1). Based on the mean comparisons, the highest H_2O_2 content was detected in the SS treatment, and the lowest was noted in the control plants (Tab. 3). The H_2O_2 content was approximately similar in four genotypes, and its accumulation severely increased in Aziziye-94, FLIP 05-77C, and *Cicer arietinum* 'Arman' and gradually increased in FLIP 00-39C under cold stress. In FLIP 03-6C, the H_2O_2 content in NS and MS treated plants was similar and then doubled with SS treatment. In *Cicer arietinum* 'Saral', the concentration of this compound under stress conditions was lower than in the control plants and was nearly equal in the MS and SS treatments. The production of H_2O_2 in ILC533 at various levels of cold stress was almost constant, and thus cold stress did not affect the H_2O_2 content. The accumulation of H_2O_2 under cold stress was much higher than in the control plants in FLIP 06-173C, and was higher in MS than in the SS treatment (Fig. 3A). Genotypes FLIP 03-6C (MS), Aziziye-94 (MS), *Cicer arietinum* 'Saral' (MS and SS), and ILC533 (SS) showed the lowest H_2O_2 production.

Discussion

The different chickpea cultivars / advanced lines employed in our study reacted differently to cold stress. The enzyme CAT removes H_2O_2 by breaking it down to form H_2O and oxygen, and oxidizes H donors with the consumption of peroxide. We have demonstrated that CAT activity increased in all the resistant, one susceptible, and one semiresistant chickpea cultivar / advanced line under cold stress, which is a similar finding to some other researchers [25–27]. *Cicer arietinum* 'Saral', the most novel genotype studied, appropriately reacted to MS and showed high CAT activity, but with intensification of cold stress, the activity of this enzyme decreased. CAT activity at both levels of cold stress in ILC533, the most susceptible advanced line, was lower than in *Cicer arietinum* 'Saral'. When an abiotic or biotic stress raises the ROS levels in plant tissues, the presence of CAT is essential for detoxification, which is important in cold stress tolerance [28,29]. SOD and CAT act as the first defense lines of the antioxidative machinery in plants. They inhibit formation of more toxic ROS and have a critical function in intracellular H_2O_2 signaling [30]. The initial activity of SOD gives rise to the production of H_2O_2; then CAT and other enzymes begin to remove H_2O_2 [31]. In the present study, genotypes with relatively high CAT activity accumulated less H_2O_2 and vice versa. In the case of cold-resistant plants, CAT is more involved in H_2O_2 detoxification than the other enzymes assayed.

In our study, the GPX activity decreased under MS compared to NS in all genotypes, except for *Cicer arietinum* 'Saral' and FLIP 00-39C. However, it decreased under SS compared to MS in all genotypes, except for ILC533 and FLIP 06-173C. In some other reports by other workers, a decreased GPX activity under cold stress was also found [26,31,32]. It can be concluded that GPX activity was more affected by cold stress in comparison to that of CAT.

APX uses ascorbate (AsA) as an electron donor for the reduction of H_2O_2 and is well known to be important in the detoxification of H_2O_2. In our study, APX activity in two susceptible genotypes grown under MS was higher than in the other treatments, but resistant and semiresistant genotypes had a different reaction under cold stress. In general, the activity of APX in chickpea leaves exposed to low temperature increased, compared to the NS control. Similar results concerning the increased activity of APX under abiotic stress conditions have been reported in different plants [33–38]. High

activity of APX can decrease the ROS levels and increase the resistance to oxidative stress, and reduced activity of this enzyme can cause the lower plant cold tolerance [31,39].

The peroxidation of polyunsaturated fatty acids (PUFA) of the plasma membrane causes MDA accumulation and oxidative damage. In many plant species, cold stress is a cause of the increase in membrane lipid peroxidation through an increase in the proportion of unsaturated plasma membrane phospholipids [13]. Our findings demonstrated an increase in the MDA content in response to cold stress in two susceptible and one semiresistant genotype. This obviously shows that oxidative stress has occurred at low temperatures, which is possibly due to the overaccumulation of ROS. Some similar results have also been reported by other workers [26,32,40,41]. In some cases, the MDA content was diminished and this drop was possibly caused by increasing CAT, SOD, APX, or GPX activities.

Hydrogen peroxide, a most abundant ROS, as a signaling molecule regulates some defensive and developmental metabolic pathways, but stress conditions may strongly raise the H_2O_2 concentration in plant tissues [42]. It can stimulate the formation of other more toxic ROS such as the hydroxyl radical [43]. However, with severe cold stress, the H_2O_2 contents of FLIP 03-6C, Aziziye-94, FLIP 05-77C, and *Cicer arietinum* 'Arman' were all nearly 2 times greater than in the control plants. These results reveal that the cold stress used in this research induced oxidative damage in the genotypes we studied. The lowest amount of H_2O_2 production under severe stress was in *Cicer arietinum* 'Saral'. Thus, the cell membrane stability of this resistant cultivar was maintained, which led to a rise in cold tolerance. In the present study, the higher H_2O_2 levels in the leaves of some chickpea genotypes exposed to low temperatures, in comparison with the unstressed plants, revealed the degree of oxidative stress during severe cold stress. Some similar results have also been reported in the literature [40,41]. High levels of H_2O_2, as detected in the severely stressed seedlings of some chickpea genotypes due to its toxicity, can create a signal which triggers the programmed cell death process [44]. The results of this research demonstrated that cold treatment of chickpea plants results in H_2O_2 accumulation that may be one of the key factors responsible for the rise in the MDA levels. Conversely, lower H_2O_2 levels, as found in the medium-stressed seedlings of some chickpea genotypes, possibly play a secondary role in the stress signaling system through prompting defensive pathways [41].

The MDA and H_2O_2 contents in *Cicer arietinum* 'Saral', a resistant cultivar, did not increase under cold stress and nearly had a decreasing trend, which may be a result of the increasing trend in antioxidant enzymes activity. The highest amount of H_2O_2 in the control plants occurred in this cultivar, which may be due to a low activity of CAT and APX. The lowest amount of H_2O_2 at the both levels of cold stress also appeared in *Cicer arietinum* 'Saral', which may also be caused by the very high activity of these two enzymes. Under severe cold stress in *Cicer arietinum* 'Saral' seedlings, a reduction in CAT and GPX activities was observed in comparison to medium stress. Reducing synthesis or increasing degradation due to ROS accumulation can reduce the activity of some enzymes in SS compared to MS treated plants [31,45].

The MDA content of FLIP 03-6C, a semiresistant advanced line, had a steady incremental trend and H_2O_2 content was doubled in SS compared to MS and NS treatments. This may be a result of the gradual decline of CAT, SOD, and APX activities under SS and the sharp decline in GPX activity under MS and SS treatments. The amount of H_2O_2 and MDA in all treatments was approximately equal in ILC533, a susceptible advanced line. The lack of any significant decline in the amount of H_2O_2 and MDA could be due to the very low activity of APX and the reduction of CAT activity under SS. This suggests that susceptible plants can also raise their ability to adapt to cold stress through various physiological and biochemical changes, but the extent of their adaptation could be variable.

The H_2O_2 content of Aziziye-94, a resistant cultivar, had an increasing trend after cold exposure, and the quantity of H_2O_2 under SS was more than twice that with NS. Furthermore, the MDA content under SS was the highest, which could be due to low activity of GPX and APX. In FLIP 00-39C, a susceptible advanced line, H_2O_2 and the MDA contents increased under cold stress, whilst the activity of CAT, APX, and SOD were high under MS and SS; this could be due to the low activity of GPX in stressed seedlings. FLIP 05-77C, a susceptible advanced line, showed a gradual enhancement

of H_2O_2 content and the MDA content was very high. This phenomenon could be due to a simultaneous reduction in the activity of CAT (gradual decrease) and GPX (severe reduction), although APX activity had an increasing trend, but its activity was lower compared to other susceptible genotypes.

In FLIP 06-173C, as a resistant advanced line, the H_2O_2 content under MS was the highest amongst other genotypes, which could be because of a susceptibility in the early stages of cold stress possibly because of a decline in GPX activity. However, by increasing the duration of cold stress, there was some resistance against oxidative stress and a reduced amount of H_2O_2 was found through enhanced activity of the antioxidant enzymes. Furthermore, the amount of MDA in this genotype was almost the same under cold stress, and this resistance could be due to high activity of antioxidant enzymes. In *Cicer arietinum* 'Arman', as a semiresistant cultivar, the H_2O_2 content showed a sharply increasing trend under cold stress, and the amount of MDA was relatively high which may result from its low activity of GPX and CAT, such that the GPX activity under SS was the lowest. At the first level of stress, CAT activity was the lowest, whereas APX had high activity under all treatment regimes, but its activity was not sufficient for effective decomposition of H_2O_2.

Conclusions

It cannot be claimed that differing reactions to cold stress are totally dependent on the genetic constitution of any plant material, but the role of genetic factors cannot be ignored. The results of our trials revealed differences in the antioxidant defense mechanisms between the various chickpea cultivars / advanced lines under cold stress, which is a crucial factor for fall chickpea cultivation in Iran. It can be concluded that the activity of antioxidant enzymes and ROS accumulation are good indicators for predicting the response of different genotypes to cold stress. The variation between chickpea genotypes in terms of cold tolerance should be analyzed further in future breeding programs.

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Wpływ długotrwałego stresu niskiej temperatury na aktywność enzymatyczną systemu antyoksydacyjnego ciecierzycy pospolitej (*Cicer arietinum* L.)

Streszczenie

Stres abiotyczny, wywołany np. zbyt niską lub wysoką temperaturą czy suszą, należy do głównych przyczyn spadku plonowania wielu roślin uprawnych. Rośliny wykształciły szereg odpowiedzi adaptacyjnych, które zapobiegają uszkodzeniom wywołanym przez wolne rodniki generowane podczas stresów środowiskowych. Celem prezentowanych badań była ocena wpływu niskiej temperatury na peroksydację lipidów i aktywność enzymów antyoksydacyjnych w liściach ośmiu odmian ciecierzycy pospolitej (*Cicer arietinum* L.). Trzytygodniowe siewki ciecierzycy uprawiane w warunkach klimatycznych Iranu eksponowano na stres niskiej temperatury (0°C) przez 24 lub 48 godzin. Określono aktywność wybranych enzymów antyoksydacyjnych. W większości genotypów stwierdzono wzrost aktywności katalazy i peroksydazy askorbinianowej oraz obniżenie aktywności peroksydazy gwajakolowej, podczas gdy aktywność dysmutazy ponadtlenkowej była przeważnie niezmieniona pod wpływem niskiej temperatury. Na podstawie uzyskanych wyników stwierdzono, że nowo wyhodowana odporna na chłód irańska odmiana ciecierzycy 'Saral' posiadała najsprawniej działający system antyoksydacyjny, podczas gdy odmiana FLIP 05-77C charakteryzowała się niską sprawnością systemu antyoksydacyjnego w warunkach chłodu.

