Cold-induced changes in antioxidant defenses and reactive oxygen species in eight wild almond species

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ABSTRACT

Introduction: Wild almond species (*Prunus* spp.) demonstrate a greater resistance to low temperatures due to their antioxidant defenses and so represent valuable germplasm sources for rootstock breeding. **Methods:** Eight genotypes of wild almond were subjected to two different cold treatments. The enzymatic and non-enzymatic antioxidant responses and the levels of some reactive oxygen species in leaves were measured. **Results:** The high ascorbate content and high ascorbate peroxidase activity found in some almond species contributed to the decrease in H_2O_2. Generally, catalase activity increased after the cold treatments, whereas superoxide dismutase activity and OH• levels varied markedly among the species. **Conclusions:** The differences in antioxidative traits among the almond species support the hypothesis of their importance in cold tolerance and could provide an useful probe to identify tolerant genotypes in breeding programs.

Keywords: α-tocopherol, Ascorbate, Antioxidant Enzymes, Cold Stress, *Prunus* Spp.

1. INTRODUCTION

Wild almond (*Prunus* spp.) demonstrate a greater resistance to abiotic and biotic stresses, including low temperatures, and so represent valuable germplasm sources for rootstock breeding.[1,2] Low temperatures induce a number of alterations in cellular components, leading to the loss of compartmentalization, reduction and impairing of photosynthesis, protein assembly and general metabolic processes.[3,4] Particularly, reactive oxygen species (ROS) produced by plants in response to cold contribute to membrane damage.[5,6] Several recent studies comparing different species have reported that chilling-resistant species have a greater antioxidant capacity than other ones that are sensitive.[4,5]

In the current study, the differences in some antioxidant enzyme activities (superoxide dismutase–SOD, ascorbate peroxidase–APX, and catalase–CAT) and in the levels of ascorbate (AsA), α-tocopherol (α-toc) and some ROS (H_2O_2 and OH•) related to chloroplastic and cellular antioxidant capabilities were examined in eight almond genotypes subjected to chilling stress.

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As almond cultivation represents the largest production of any commercial tree nut product and it supports the economies of many tropical countries, it is important to study and select almond genotypes with the ability to endure environmental constraints in order to ensure the viability of almond in cold conditions.

2. MATERIALS AND METHODS

The eight wild almond species (genus *Prunus*, subgenus *Amygdalus*) here studied were: *P. communis* [L. (Archangeli)], *P. eleaegnifolia* (Mill.), *P. orientalis* (Mill.) [syn. *P. argentia* (Lam)], *P. lycioides* (Spach), *P. reuteri* (Bioss & Bushe) [syn. *P. hordia* (Spach)], *P. arabica* (Olivier), *P. glauca* (Browick), and *P. scoparia* (Spach). Field expeditions were carried out in 2010 with the aim of collecting wild almond species throughout Iran.[1,2] Two-year old trees were planted in pots in September 2011 and then gradually transplanted three times to bigger pots to stimulate root development. The soil consisted of 22% topsoil, perlite, and wood mulch each, 17% vermiculite, 11% peat mulch and 6% sand. Successively, potted plants were transferred into growth chambers (12 h photoperiod, 65% relative humidity, 800 µmol m^{-2} s^{-1} irradiance) and submitted (a) to a gradual temperature decrease (0.5°C d^{-1}) from 25/20°C to 15/10°C (day/night) over 24 d [15/10°C], to allow the expression of acclimation ability and (b) to a 3-d chilling cycle, where the plants were subjected to 4°C during the night and in the first 4 h of the morning, followed by a gradual rise up to 15°C applied throughout the rest of the diurnal period [3 × (15°C/4°C)]. Subsequently, chilled plants from treatment (b) were allowed to recover at 25/20°C for 7 d. Plants maintained at 25/20°C throughout the experiment were considered as controls.

Total SOD (EC 1.15.1.11) and CAT (EC 1.11.1.6) activities in leaves were measured according to Sofo et al.,[7] whereas ascorbate peroxidase (EC 1.11.1.11) activity was assayed according to Sofo et al.[8] The soluble protein content was determined by the Coomassie blue dye binding method. The extraction and estimation of total AsA was carried out in leaves according to Sofo et al.[8] Foliar levels of α-toc, H_2O_2 and hydroxyl radical (OH•) were measured according to Murata et al.[9]

The experiments were conducted using a randomized complete block design with ten replications ($n = 10$). The data were analyzed using a Statistical Analysis Software Version 9.1 (SAS Institute Inc.; Cary, NC, USA). Differences in studied traits were determined by the analysis of variance (ANOVA) followed by a Tukey's range test for mean comparisons ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Antioxidant enzyme activities

In this work, SOD activity showed significant changes after [15/10°C] in *P. communis* (+79%, compared to the controls), *P. eleagnifolia* (+59%), *P. reuteri* (+276%) and *P. scoparia* (–71%) (Table 1). Under [3 × (15°C/4°C)], *P. communis*, *P. reuteri*, *P. arabica* and *P. glauca* showed significant increases (+311%, +255%, +213% and +57%, respectively), *P. eleagnifolia* maintained its activity, while *P. orientalis*, *P. lycioides* and *P. scoparia* had significant declines to more than half of the controls (Table 1). Upon recovery from [3 × (15°C/4°C)], *P. eleagnifolia* and *P. glauca* tended toward the controls, while *P. communis, P. reuteri* and *P. arabica* maintained significant higher SOD activities (+164%, +267% and +143%, respectively) (Table 1). In contrast, *P. orientalis*, *P. lycioides* and *P. scoparia* showed an opposite trend under recovery conditions (approximately one-third of controls) (Table 1).

Significant changes in APX activity were detected among some genotypes after cold treatments (Table 1). In particular, the potential for H_2O_2 removal by APX was continuously enhanced in *P. eleagnifolia*, *P. arabica*, *P. glauca* and *P. scoparia*, with activities doubled at the end of [15/10°C] and maintained at the same level after [3 × (15°C/4°C)] (Table 1). Upon recovery, the same almond genotypes showed small increases in relation to the controls (Table 1). Interestingly, *P. arabica* and *P. glauca* showed parallel significant increases in SOD and APX activities after chilling (Table 1), suggesting that APX appeared to play an essential protective role in the scavenging processes when coordinated with SOD activity,[10] as APXs are chloroplastic or cytosolic enzymes which scavenged H_2O_2 generated primarily through SOD action.[10]

Catalase are able to dismutase H_2O_2 into water and O_2, whereas APX utilizes AsA as an electron donor in the neutralization of H_2O_2.[11] During [15/10°C], CAT activity increased except in *P. lycioides* and *P. scoparia*, with the higher peak of CAT activity in *P. communis* (+107%) (Table 1). However, after exposure to [3 × (15°C/4°C)], all the species showed decreased activity of their control activities, even if significantly only in *P. communis* and *P. eleagnifolia* (–38% and –56%, respectively) (Table 1). Upon recovery, all the wild almond species studied presented increases in CAT activities, reaching particularly high values in *P. communis* (+269%) (Table 1). Thus, it appears that the up-regulation of CAT activity is an important part of the response in *Prunus* species to

Table 1. Activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) in leaves of the eight wild almond species.

Treatment	SOD activity (units mg^{-1} protein)							
	P. communis	*P. eleagnifolia*	*P. orientalis*	*P. lycioides*	*P. reuteri*	*P. arabica*	*P. glauca*	*P. scoparia*
25/20°C (control)	11.7 ± 0.25 c	13.1 ± 0.26 c	29.7 ± 0.31 a	32.6 ± 0.25 a	28.6 ± 0.19 b	9.1 ± 0.38 b	18.6 ± 0.26 b	96.6 ± 0.42 a
[15/10°C]	20.9 ± 0.22 b	20.8 ± 0.28 a	21.2 ± 0.28 a	23.6 ± 0.28 a	32.3 ± 0.33 a	11.7 ± 0.40 b	20.4 ± 0.25 b	28.3 ± 0.38 b
[3 × (15°C/4°C)]	48.1 ± 0.24 a	11.5 ± 0.21 c	8.9 ± 0.19 b	14.5 ± 0.31 b	30.5 ± 0.30 a	28.5 ± 0.31 a	29.2 ± 0.31 a	30.1 ± 0.32 b
25/20°C (recovery)	30.9 ± 0.32 b	10.7 ± 0.30 c	8.6 ± 0.18 b	9.7 ± 0.24 c	31.6 ± 0.29 a	22.1 ± 0.40 a	19.9 ± 0.28 b	30.5 ± 0.27 b
Treatment	APX activity (units mg^{-1} protein)							
	P. communis	*P. eleagnifolia*	*P. orientalis*	*P. lycioides*	*P. reuteri*	*P. arabica*	*P. glauca*	*P. scoparia*
25/20°C (control)	7.6 ± 1.45 a	7.5 ± 0.89 a	7.7 ± 1.15 b	8.5 ± 1.77 b	8.1 ± 1.23 b	7.3 ± 1.74 b	10.2 ± 1.47 b	12.6 ± 1.47 c
[15/10°C]	8.5 ± 1.22 a	17 ± 1.14 b	8.1 ± 1.65 a	7.6 ± 1.35 b	7.5 ± 0.96 b	14.5 ± 1.10 a	20.6 ± 2.44 a	34.5 ± 2.17 a
[3 × (15°C/4°C)]	9.1 ± 1.12 a	14.5 ± 0.72 b	9.3 ± 1.22 a	10.1 ± 1.19 a	11.2 ± 1.08 a	19.7 ± 1.28 a	27.6 ± 2.14 a	31.7 ± 2.10 a
25/20°C (recovery)	9.4 ± 1.34 a	12.5 ± 1.33 b	8.6 ± 1.44 a	7.6 ± 0.98 b	8.4 ± 0.84 b	8.2 ± 1.87 b	22.1 ± 1.87 a	22.4 ± 1.88 b
Treatment	CAT activity (units mg^{-1} protein)							
	P. communis	*P. eleagnifolia*	*P. orientalis*	*P. lycioides*	*P. reuteri*	*P. arabica*	*P. glauca*	*P. scoparia*
25/20°C (control)	18.5 ± 0.02 b	48.5 ± 0.44 c	70.6 ± 0.35 c	42.5 ± 0.87 b	34.4 ± 0.47 c	60.4 ± 1.22 c	71.3 ± 1.20 c	89.4 ± 0.65 b
[15/10°C]	17.6 ± 0.23 b	84.3 ± 0.38 b	103.4 ± 1.25 b	32.8 ± 0.97 b	51.6 ± 1.12 b	90.4 ± 1.77 b	108.7 ± 1.07 b	81.5 ± 0.45 b
[3 × (15°C/4°C)]	5.3 ± 0.48 d	21.5 ± 0.25 d	56.7 ± 1.44 c	34.3 ± 1.58 b	32.7 ± 0.85 c	52.7 ± 2.14 c	63.2 ± 0.87 c	72.3 ± 0.38 b
25/20°C (recovery)	31.4 ± 0.35 a	134.8 ± 0.74 a	189.6 ± 1.42 a	51.4 ± 1.87 a	58.8 ± 1.75 a	118.6 ± 2.44 a	201.3 ± 1.25 a	178.5 ± 0.84 a

Plants were submitted to a gradual temperature decrease (0.5°C d–1) from 25/20°C to 15/10°C (day/night) over 24 d [15/10°C], or to a 3-d chilling cycle, where the plants were subjected to 4°C during the night and in the first 4 h of the morning, followed by a gradual rise up to 15°C applied throughout the rest of the diurnal period [3 X (15°C/4°C)]. Chilled plants from treatment [3 X (15°C/4°C)] were allowed to recover at 25/20°C for 7 d (recovery). Plants maintained at 25/20°C throughout the experiment were considered as controls (control). Each value represents the mean (n = 10) ± standard error. The different letters after the numbers indicates significant differences between treatments (within the same column, for each parameter) at P < 0.05, according to Tukey's range test for mean comparisons.

cold. Similar results have been observed in some chilling tolerant species.[11] Catalase has a high protein turnover and is inhibited by light and O_2, thus requiring continuous synthesis that could be compromised under stress conditions.[3,4,11] The increases of CAT activity after [15/10°C] suggest a higher cold tolerance status in cold acclimated plants, whose leaf CATs are better protected against photo-inactivation and synthesis is less suppressed by sudden temperature drops.[11]

3.2 Levels of non-enzymatic antioxidants and ROS

Ascorbate is a potent ROS scavenger that reacts with H_2O_2 in a reaction catalyzed by APX, and non-enzymatically with $^1O_2$, O_2^-, OH• and lipid hydroperoxides.[10] Cold conditions (15/10°C) had a different impact on the AsA content of wild almond genotypes, with significant increases in *P. communis*, *P. eleagnifolia, P. glauca* and *P. scoparia* (Table 2). Particularly, after [15/10°C], *P. communis* doubled its AsA level (Table 2). After [3 × (15°C/4°C)], *P. eleagnifolia, P. lyciodes* and *P. reuteri* showed the highest AsA increases (+81%, +94% and +139%, respectively, compared to the controls) (Table 2). Upon recovery, all almond genotypes, except *P. communis, P. orientalis*, *P. arabica* and *P. scoparia* maintained significant higher AsA levels (Table 2). Noteworthy, the higher ASA levels after [3 × (15°C/4°C)] observed in all the species (Table 2) were positively correlated with the high values of APX activity (Table 1). This is particularly evident in *P. eleagnifolia*, that showed the highest ASA peak (Table 2) paralleled by a two-fold increase in APX activity (Table 1). The increase in *a*-toc content is characteristic of stress-tolerant species, as it deactivates $^1O_2$, O_2^-, OH• and partly H_2O_2, and limits lipid peroxidation by reducing lipid peroxyl radicals, helping also to preserve an adequate redox state in chloroplasts and stabilizing membrane structures due to its interactions with

Table 2. Levels of ascorbate and α-tocopherol in leaves of the eight wild almond species.

Treatment	Ascorbate (mmol g^{-1} fw)							
	P. communis	*P. eleagnifolia*	*P. orientalis*	*P. lycioides*	*P. reuteri*	*P. arabica*	*P. glauca*	*P. scoparia*
25/20°C (control)	152.2 ± 2.33 c	232.4 ± 1.68 c	183.7 ± 1.25 b	283.5 ± 2.15 c	215.3 ± 1.18 c	156.6 ± 1.08 b	164.1 ± 1.14 b	235.7 ± 1.12 b
[15/10°C]	320.2 ± 2.47 a	357.3 ± 2.14 b	192.3 ± 1.74 b	332 ± 1.88 c	247.5 ± 2.44 c	182.8 ± 0.85 b	234.5 ± 2.01 a	348.5 ± 0.85 a
[3 × (15°C/4°C)]	256.8 ± 1.72 b	421.2 ± 2.17 a	274.4 ± 0.98 a	551.2 ± 2.84 a	514.9 ± 2.36 a	238.8 ± 1.48 a	259.6 ± 1.47 a	257.0 ± 0.57 b
25/20°C (recovery)	160.8 ± 1.35 c	453.8 ± 2.38 a	152.7 ± 1.44 b	424.1 ± 2.44 b	355.6 ± 1.28 b	184.9 ± 0.98 b	254.1 ± 2.33 a	191.9 ± 1.17 c
Treatment	α-tocopherol (mmol g^{-1} fw)							
	P. communis	*P. eleagnifolia*	*P. orientalis*	*P. lycioides*	*P. reuteri*	*P. arabica*	*P. glauca*	*P. scoparia*
25/20°C (control)	25.2 ± 0.05 c	21.3 ± 0.43 c	28.6 ± 0.68 c	24.2 ± 0.06 c	17.1 ± 0.14 c	34 ± 1.20 b	44.3 ± 0.02 b	54.3 ± 0.04 a
[15/10°C]	54.5 ± 0.04 a	30.0 ± 0.31 b	47.9 ± 0.85 a	25.6 ± 0.08 c	23.7 ± 0.34 b	25.6 ± 0.87 c	35.5 ± 0.35 c	32.8 ± 0.35 b
[3 × (15°C/4°C)]	36.8 ± 0.02 b	44.7 ± 0.02 a	35.4 ± 1.02 b	51.9 ± 0.05 b	43.4 ± 0.05 a	44.8 ± 0.57 a	53.9 ± 0.05 a	41.8 ± 0.06 a
25/20°C (recovery)	55.2 ± 0.48 a	36.0 ± 0.35 b	55.3 ± 0.65 a	61.5 ± 1.04 a	36.5 ± 0.04 a	23.5 ± 0.65 c	34.1 ± 0.14 c	21.6 ± 0.47 c

Plants were submitted to a gradual temperature decrease (0.5°C d–1) from 25/20°C to 15/10°C (day/night) over 24 d [15/10°C], or to a 3-d chilling cycle, where the plants were subjected to 4°C during the night and in the first 4 h of the morning, followed by a gradual rise up to 15°C applied throughout the rest of the diurnal period [3 X (15°C/4°C)]. Chilled plants from treatment [3 X (15°C/4°C)] were allowed to recover at 25/20°C for 7 d (recovery). Plants maintained at 25/20°C throughout the experiment were considered as controls (control). Each value represents the mean (n = 10) ± standard error. The different letters after the numbers indicates significant differences between treatments (within the same column, for each parameter) at P < 0.05, according to Tukey's range test for mean comparisons.

polyunsaturated fatty acyl chains.[3,4,8] Here, *P. communis* and *P. orientalis* showed the greatest increases in α-toc content after [15/10°C] (+116% and +67%, respectively) (Table 2). Subjected to [3 × (15°C/4°C)], all the almond genotypes showed higher values of α-toc, with the exception of *P. scoparia* (–33%) (Table 2). Upon recovery condition, *P. communis*, *P. lycioides,* and *P. reuteri* showed the highest significant increases (Table 2).

The almond species showed different tendencies regarding H_2O_2 content during [15/10°C]: *P. lycioides* and *P. scoparia* had the levels of H_2O_2 significantly decreased (–65% and –33%, respectively, compared to the controls), while the remaining species showed significant increases (Table 3). The low H_2O_2 levels in *P. lycioides* and *P. scoparia* were maintained also after [3 × (15°C/4°C)]. By contrary, *P. reuteri* and *P. arabica* showed significantly high values (Table 3). After recovery, *P. scoparia* maintained significant low H_2O_2 content, while no differences with the controls were detected for the other species (Table 3). After cold exposure, OH• levels varied markedly among wild almond species (Table 3). The [15/10°C] treatment provoked no effects on OH• content, whereas [3 × (15°C/4°C)] caused significant decreases in *P. eleagnifolia* and *P. scoparia* (–41% and –42%, respectively), likely due to the increase in their anti-oxidative components (Tables 1 and 2), according to Thomashow[3] and Xin et al.[4] Significant OH• increases were observed in the remaining species (Table 3). Some of the *Prunus* species showed significant increases in OH• levels after recovery, with *P. communis, P. lycioides, P. reuteri,* displaying increases of more than two-fold (Table 3).

3.3 Conclusions

From the overall analysis of the results, *Prunus communis* and *P. reuteri*, and partly *P. arabica* and *P. glauca*, showed the greatest ability to adapt to cold-caused oxidative stress by enzymatic and non-enzymatic antioxidant defenses as they showed rapid and concomitant recovery of SOD, APX and CAT, ASA and α-toc after chilling stress (Tables 1 and 2). It is noteworthy that, instead of dramatic changes in a single component, a moderate and generalized enhancement of antioxidant molecules was found during both the cold treatments. This reflects the presence of a highly integrated and regulated system that scavenges and detoxifies ROS, comprising several enzymes and non-enzyme molecules. The antioxidative network found in wild almond can constitute an useful probe to the identification of cold-tolerant species and to the improvement of rootstock for almond breeding and cultivation under low temperature environments.

REFERENCES

1. Sorkheh K, Shiran B, Rouhi V, Asadi E, Jahanbazi H, Moradi H, Gradziel TM, Martinez Gomez P. Phenotypic diversity within native Iranian almond (*Prunus* sp.) species and their breeding potential. Gen Resour Crop Evol 2009; 56:947–961.

Table 3. Levels of hydrogen peroxide (H_2O_2) and hydroxyl radical (OH•) in leaves of the eight wild almond species.

Treatment	H_2O_2 (µmol g^{-1} fw)							
	P. communis	*P. eleagnifolia*	*P. orientalis*	*P. lycioides*	*P. reuteri*	*P. arabica*	*P. glauca*	*P. scoparia*
25/20°C (control)	413.2 ± 1.02 a	376.3 ± 0.54 b	356.6 ± 1.84 b	267.8 ± 1.04 a	364.9 ± 0.67 b	320.5 ± 0.68 b	310.5 ± 1.20 b	406.4 ± 0.54 a
[15/10°C]	312.6 ± 1.25 b	480.5 ± 0.48 a	436.5 ± 1.02 a	94.6 ± 0.57 c	435.5 ± 0.54 a	408.6 ± 0.47 a	385.6 ± 1.32 a	273.5 ± 0.68 b
[3 × (15°C/ 4°C)]	331.3 ± 2.01 b	348.3 ± 0.68 b	237.3 ± 0.85 c	125.5 ± 0.68 b	434 ± 0.45 a	404.8 ± 1.02 a	248.4 ± 0.85 c	201.6 ± 0.35 c
25/20°C (recovery)	475.8 ± 0.65 a	327.4 ± 1.02 b	298.2 ± 0.25 b	262.3 ± 1.84 a	310.5 ± 0.86 b	329.6 ± 1.84 b	283.8 ± 0.42 b	176.4 ± 0.24 d
Treatment	OH• (µmol g^{-1} fw)							
	P. communis	*P. eleagnifolia*	*P. orientalis*	*P. lycioides*	*P. reuteri*	*P. arabica*	*P. glauca*	*P. scoparia*
25/20°C (control)	20.3 ± 0.05 c	121.2 ± 1.05 a	89.3 ± 0.32 b	38.4 ± 0.25 b	49.35 ± 0.38 b	108.6 ± 0.48 b	111.3 ± 0.65 b	148.3 ± 0.68 a
[15/10°C]	21.6 ± 0.04 c	107.5 ± 1.04 a	110.5 ± 0.25 b	40.8 ± 0.84 b	35.14 ± 0.52 b	90.6 ± 0.05 b	102.2 ± 1.02 b	132.6 ± 0.85 a
[3 × (15°C/ 4°C)]	125.8 ± 0.52 a	71.4 ± 0.87 c	117.3 ± 0.47 a	104.5 ± 0.35 a	92.35 ± 0.35 a	163.6 ± 0.06 a	147.3 ± 1.04 a	86.4 ± 0.04 b
25/20°C (recovery)	48.9 ± 0.07 b	100.8 ± 0.48 a	88.4 ± 0.35 b	102.3 ± 0.65 a	107.63 ± 0.48 a	143.2 ± 0.57 a	129.8 ± 0.04 a	130.2 ± 0.03 a

Plants were submitted to a gradual temperature decrease (0.5°C d–1) from 25/20°C to 15/10°C (day/night) over 24 d [15/10°C], or to a 3-d chilling cycle, where the plants were subjected to 4°C during the night and in the first 4 h of the morning, followed by a gradual rise up to 15°C applied throughout the rest of the diurnal period [3 X (15°C/4°C)]. Chilled plants from treatment [3 X (15°C/4°C)] were allowed to recover at 25/20°C for 7 d (recovery). Plants maintained at 25/20°C throughout the experiment were considered as controls (control). Each value represents the mean (n = 10) ± standard error. The different letters after the numbers indicates significant differences between treatments (within the same column, for each parameter) at P < 0.05, according to Tukey's range test for mean comparisons.

2. Sorkheh K, Shiran B, Rouhi V, Khodambashi M, Sofo A. Regulation of the ascorbate–glutathione cycle in wild almond during drought stress. Russ J Plant Physiol, 2011; 58:76–84.
3. Thomashow MF. Plant Cold Acclimation: freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Physiol Plant Mol Biol, 1999; 50: 571–599.
4. Xin Z, Browse I. Cold comfort farm: the calumniation of plants to freezing temperature. Plant Cell Environ,
5. Pukacki PM, Kamińska-Rożek E. Reactive species, antioxidants and cold tolerance during deacclimation of *Picea abies* populations. Acta Physiol Plant, 2013; 35:129–138.
6. Nazari M, Maali Amiri R, Mehraban FH, Khaneghah HZ. Change in antioxidant responses against oxidative damage in black chickpea following cold acclimation. Russ J Plant Physiol, 2012; 59:183–189.
7. Sofo A, Dichio B, Xiloyannis C, Masia A. Effects of different irradiance levels on some antioxidant enzymes and on malondialdehyde content during rewatering in olive tree. Plant Sci, 2004; 166:293–302.
8. Sofo A, Tuzio AC, Dichio B, Xiloyannis C. Influence of water deficit and rewatering on the components of the Ascorbate–Glutathione cycle in four inter-specific *Prunus* hybrids. Plant Sci, 2005; 169: 403–412.
9. Murata N, Allakhverdiev SI, Nishiyama Y. The mechanism of photoinhibition in vivo: re-evaluation of the roles of catalase, α-tocopherol, non-photochemical quenching, and electron transport. Biochim Biophys Acta, 2012; 1817:1127–1133.
10. Mhamdi A, Queval G, Chaouch S, Vanderauwera S, Van Breusegem F, Noctor G. Catalase function in plants: a focus on *Arabidopsis* mutants as stress-mimic models. J Exp Bot, 2010; 61:4197–4220.
11. Shang W, Schmidt M, Feierabend J. Increased capacity for synthesis of the D1 protein and of catalase at low temperature in leaves of cold-hardened winter rye (*Secale cereale* L.). Planta, 2003; 216:865–873.