ISSN 1021-4437, Russian Journal of Plant Physiology, 2011, Vol. 58, No. 1, pp. 76–84. © Pleiades Publishing, Ltd., 2011.

RESEARCH PAPERS

Regulation of the Ascorbate–Glutathione Cycle in Wild Almond during Drought Stress[1]

K. Sorkheh[a], B. Shiran[a], V. Rouhi[a], M. Khodambashi[a], and A. Sofo[b]

[a] *Department of Agronomy and Plant Breeding, Faculty of Agriculture, Shahrekord University, P.O. Box 115, Shahrekord, Iran*

[b] *Dipartimento di Scienze dei Sistemi Colturali, Forestali e dell' Ambiente, Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy;*

fax: +39-09-71-20-5378; e-mail: adriano.sofo@unibas.it

Received April 12, 2010

Abstract—In wild species of almond (*Prunus* spp.), the activities of ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR), as well as the levels of ascorbate/glutathione pools and H_2O_2 were subjected to water deficit and shade conditions. After 60 days of water shortage, the species were subjected to a rewatering treatment. During water recovery, leaves exposed to sunlight and leaves under shade conditions of about 20–35% of environmental irradiance were sampled. After 70 days without irrigation, mean predawn leaf water potential of all the species fell from −0.32 to −2.30 MPa and marked decreases in CO_2 uptake and transpiration occurred. The activities of APX, MDHAR, DHAR, and GR increased in relation to the severity of drought stress in all the wild species studied. Generally, APX, MDHAR, DHAR, and GR were down-regulated during the rewatering phase and their activities decreased faster in shaded leaves than in sun-exposed leaves. The levels in total ascorbate, glutathione, and H_2O_2 were directly related to the increase in drought stress and subsequently decreased during rewatering. The antioxidant response of wild almond species to drought stress limits cellular damage caused by reactive oxygen species during periods of water deficit and may be of key importance for the selection of drought-resistant rootstocks for cultivated almond.

Keywords: *Prunus* spp, antioxidant enzymes, ascorbate–glutathione cycle, ascorbate peroxidase, dehydroascorbate reductase, glutathione reductase, monodehydroascorbate reductase, oxidative stress, water deficit.

DOI: 10.1134/S1021443711010201

INTRODUCTION

The genus *Prunus* belongs to the family Rosaceae and comprises more than 400 species adapted to temperate areas and cultivated in Asia and Europe. Stone fruit crops, such as almond, peach, plum, apricot, and cherry, are typical and economically important cultures mainly localized in Mediterranean regions, where the spring–summer period is often characterized by high temperatures, high irradiance levels, and the lack of precipitation. In particular, almond (genus *Prunus*, subgenus *Amygdalus*) is the most important tree nut produced on a global basis, and its limited gene pool limits the cultivation to specific areas with Mediterranean climate [1, 2].

Drought stress in plants, especially in combination with high levels of irradiance, is associated with increased levels of reactive oxygen species (ROS), by-products of aerobic metabolism that are produced through the disruption of the electron transport system and oxidizing metabolic activities [3]. Excessive levels of ROS are toxic for plant cells due to their oxidative damage to cellular structures and macromolecules, which in turn causes photoinhibition of the photosynthetic apparatus [4]. ROS also may play a positive role because some of them, in particular $O_2^{\bullet -}$ and H_2O_2, act as signal molecules during drought stress responses and trigger defense responses [4]. In plants under well-watered conditions, ROS are efficiently eliminated by several antioxidant compounds, whereas during drought conditions ROS production exceeds the capacity of the antioxidant systems to remove them [3].

The antioxidant enzymes of the ascorbate–glutathione cycle of plants operate both in the chloroplasts and cytosol and include ascorbate peroxidase (APX, EC 1.11.1.11), monodehydroascorbate reductase (MDHAR, EC 1.6.5.4), dehydroascorbate reductase (DHAR, EC 1.8.5.1), and glutathione reductase (GR, EC 1.6.4.2). Ascorbate and glutathione, two antioxidants within the ascorbate–glutathione cycle

[1] This text was submitted buy the authors in English.

Abbreviations: APX—ascorbate peroxidase; AsA—ascorbate; DHA—dehydroascorbate; DHAR—dehydroascorbate reductase; GR—glutathione reductase; GSH—glutathione; GSSG—glutathione disulfide; LWP—leaf water potential; MDHA—monodehydroascorbate; MDHAR—monodehydroascorbate reductase; PAR—photosynthetic active radiation.

with a non-enzymatic action, are able to detoxify H_2O_2 and are located both within the cell and in the apoplast [5]. Ascorbate (AsA), synthesized on the inner membrane of the mitochondria, reacts chemically with $^1O_2$, $O_2^{\cdot -}$, $HO^{\cdot}$, and thiol radical and acts as the natural substrate of many plant peroxidases [5]. Glutathione (GSH), the substrate of DHAR, is a tripeptide synthesized in the cytosol and chloroplasts, which scavenges $^1O_2$ and H_2O_2 and is oxidized to glutathione disulfide (GSSG), when it acts as an antioxidant and redox regulator [6].

The response to water deficit of almond trees is a well documented process [7–10], but only few studies highlighted the importance of antioxidant enzymes in cultivated fruit trees [11–13] and no studies on antioxidant capability of almond have been hitherto performed. Wild almond species demonstrate a greater resistance to abiotic and biotic stresses and so represent valuable germplasm sources for rootstock breeding, especially under non-irrigation conditions [14]. Therefore, the aim of this work was to study the changes of antioxidant enzyme activities and the level of some compounds involved in the ascorbate–glutathione cycle in drought-stressed plants of eight wild almond species from different geographical points of Iran. Antioxidant and physiological responses were also studied during the following rewatering phase in leaves exposed to two different irradiance levels, in order to study the contribution of light to oxidative stress. The wild almond species here studied may be potential sources for resistance of cultivated almond to drought stress as well as for modified tree and nut traits.

MATERIALS AND METHODS

Plant material, collecting regions, and experimental design. The wild almond species studied belonging to the genus *Prunus,* subgenus *Amygdalus,* were *P. communis* (L.) Archang, *P. elaeagnifolia* (Spach) Fritsch, *P. orientalis* Mill. (syn. *P. argentea* Lam.) in the section Euamygdalus Spach; *P. lycioides* Spach, *P. reuteri* Boiss. et Bushe in the section Lycioides Spach; and *P. arabica* (Olivier) Neikle, *P. glauca* (Browicz) A.E. Murray, *P. scoparia* Spach in the section Spartioides Spach. The number of accessions sampled per site ranged from one to five, depending on habitat diversity and availability at collection time.

Field expeditions were carried out in 2005 and 2006. Sites were selected based on previous recent literature [15, 16], indigenous information, or conspicuous presence. The experimental scheme was carried out using wild species of almond in spring 2009, spaced at 1 m in the row with 1 m between rows and grew uniformly outdoors in 5.0-m^3 containers filled with a silty-clay loam. Plants were divided into the two groups: drought-stressed plants and control plants not subjected to drought stress. The control plants were maintained under optimal soil water conditions (85% of the field water capacity) during the whole 84-day experimental period, whereas drought-stressed plants were subjected to a water shortage period starting from July 10 to September 18. Containers were covered with plastic film in order to avoid rainfall infiltrations and evaporation from the soil surface. After the 70-day drought period, stressed plants were subjected to a rewatering treatment for 16 days (from September 19 to October 4), while control plants continued to be kept under optimal soil water conditions.

Environmental parameters, plant gas exchange, and water status. For each day of the experimental period, measurements of air temperature and relative humidity were taken by a data logger at Chaharmahal & Bakhtiari, Metrological Administration (www.chaharmahalmet.ir, Shahrekord, Iran) located inside the experimental plot. Vapor pressure deficit (VPD) was calculated from the values of air temperature and relative humidity at 11:00 a.m., according to Goudriaan and van Laar [17].

Three drought-stressed plants and three control plants of each species were chosen to measure physiological parameters at 0, 13, 23, 70, and 84 days from the beginning of the experimental period using four fully expanded leaves selected from each plant along the median segment of new-growth shoots and marked at the beginning of the experiment. The measurements of CO_2 uptake and transpiration were carried out using a programmable, an open-flow gas exchange LI-6400 portable system (Li-Cor, United States) operated at 500 μmol/s flow rate, under field conditions at 9:30. The measurements of leaf water potential (LWP) were carried out predawn (at 4:00) at 0, 13, 23, 70, and 84 days from the beginning of the experimental period on three fully expanded leaves selected from each plant along the median segment of new-growth shoots using a pressure chamber (Weiss Umwelttechnique CMBH, D-6301 Lindenstruth, Germany).

Leaf sampling. Three drought-stressed plants having similar LWP and three control plants from each species were selected in each date for tissue sampling. Leaves were collected at 0, 13, 23, 70, and 84 days from the beginning of the drought-stress period. Each sample contained three fully expanded leaves taken along the median segment of new-growth shoots and fully exposed to sunlight (photosynthetically active radiation (PAR) range under clear sky = 1600–1800 μmol/(m^2 s) at 12:00]. At the end of the rewatering phase, both in rewatered and control plants, two types of leaf samples were collected: the former with leaves exposed to sunlight and the latter with leaves picked in the inner part of the canopy in shade conditions of about 50% of environmental PAR (PAR range = 800–900 μmol/(m^2 s) at 12:00). Light levels were followed by the LI-6400 external quantum light sensor. Leaf samples were washed with distilled water, dried with filter paper,

immediately frozen in liquid nitrogen, and then stored at −80°C.

Enzyme activities and total soluble protein. All procedures for enzyme extraction and determination of enzyme activities were carried out at 0°C on ice bath unless otherwise stated. A 1.0 g aliquot of leaves was homogenized in 10 ml of 50 mM potassium phosphate buffer, pH 7.6, containing 1% (w/v) polyvinylpolypyrrolidone (PVPP) and 1 mM EDTA. The homogenate was filtered through three layers of cheesecloth (Miracloth) and then centrifuged at 15000 *g* for 30 min at 4°C. The obtained supernatant was recovered, desalted on a Sephadex^{TM} G-25M column, and used for the enzyme activity assays.

APX activity was measured spectrophotometrically by recording a decrease in the ascorbate content at 290 nm, according to Sofo et al. [12]. MDHAR activity was tested after the method of Foyer et al. [18] by following a decrease in absorbance at 340 nm due to NADH oxidation. DHAR activity was determined by monitoring the increase in absorbance at 265 nm due to AsA production, according to Sofo et al. [13]. GR activity was measured by following a decrease in absorbance at 340 nm due to NADPH oxidation after the method of Carlberg and Mannervik [19].

The total soluble protein was determined according to Smith et al. [20], using BSA as a calibration standard. All enzyme activities were expressed as units per milligram of total soluble proteins.

Ascorbate, glutathione, and H_2O_2 levels. A 0.5 g aliquot of leaves was homogenized in 1.0 ml of ice-cold 2.5 N $HClO_4$. The homogenate was filtered through three layers of cheesecloth (Miracloth) and then centrifuged at 15000 *g* for 5 min. The supernatant was neutralized with 5 M K_2CO_3 to pH 4.5 for ascorbate determination and to pH 6.5 for glutathione determination. Ascorbic acid (AsA) was measured spectrophotometrically by reading absorbance at 265 nm due to ascorbate oxidation by ascorbate oxidase, according to the method Foyer et al. [18]. The concentration of dehydroascorbate (DHA) was calculated as the difference between total ascorbate and AsA. The levels of glutathione were measured spectrophotometrically by monitoring the reduction of 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) at 412 nm, after the method of Griffith [21]. The concentration of reduced glutathione (GSH) was calculated as the difference between total glutathione and GSSG. H_2O_2 determination was carried out according to the method of Lee and Lee [22].

Statistical analysis. The values of gas exchange parameters were represented as means of 12 measurements from three selected plants (four measurements per plant and three replications of each measurement), whereas the values of LWP were represented as means of nine measurements from three selected plants (three measurements per plant and three replications of each measurement). Enzyme activities were expressed as means of three measurements from three plants having a similar value of LWP (one measurement per plant and three replications of each measurement). Statistical analysis was performed using analysis of variance (ANOVA). Significant differences were determined at $P \leq 0.05$, according to Fisher's LSD tests.

RESULTS

Environmental Conditions and Plant Physiological Parameters

The highest value of air temperature was 35.2°C after 15 days from the beginning of the drought period. The mean values of all the daily values of air temperature and relative humidity were 28.6°C and 65.2%, respectively. VPD range was between 2.5 and 0.3 kPa, with a mean value of 0.8 kPa. The water shortage during the drought period was paralleled by a substantial decrease in the leaf LWP in all the species studied, starting from 23 days after the beginning of the drought phase (Fig. 1a). In particular, *P. orientalis* and *P. arabica* reached the lowest values of LWP (mean minimum value of about −2.00 MPa after 70 days). In drought-stressed plants, the values of CO_2 uptake and transpiration decreased during the drought period in all the species studied, reaching the lowest values at 23 and 70 days from the beginning of the drought period (Figs. 1b, 1c). During the recovery period, at 84 days from the beginning of the experimental period, LWP, CO_2 uptake, and transpiration values recovered in all the species tested (Fig. 1). In control plants, the values of LWP, CO_2 uptake, and transpiration remained relatively stable throughout the experimental period.

Antioxidant Enzyme Activities and Ascorbate/Glutathione Pools during the Drought Period

APX activity showed an increase starting from 13 days after the beginning of drought in all the species tested (Fig. 2a). In particular, during the last dates of the drought phase, *P. communis, P. orientalis, P. glauca,* and *P. scoparia* manifested the highest APX activity, as compared to the other almond species. The values of MDHAR activity remained stable in all the species during the first 23 days of the experimental period and then increased at severe drought stress (Fig. 2b). *P. lycioides* and *P. glauca* had the highest values of MDHAR activity among all the wild species of almond studied (Fig. 2b). DHAR activity was directly related to drought stress levels in all the species studied, showing a gradual increase, starting after 13 days from the beginning of the water deficit (Fig. 2c). In particular, *P. communis, P. orientalis*, and *P. lycioides* showed higher values of DHAR activity as compared to the other almond species. GR activity, especially for *P. arabica* and *P. scoparia,* showed a sharp increase with increasing drought stress in all the almond species studied, with a plateau between 23 and 70 days (Fig. 2d).

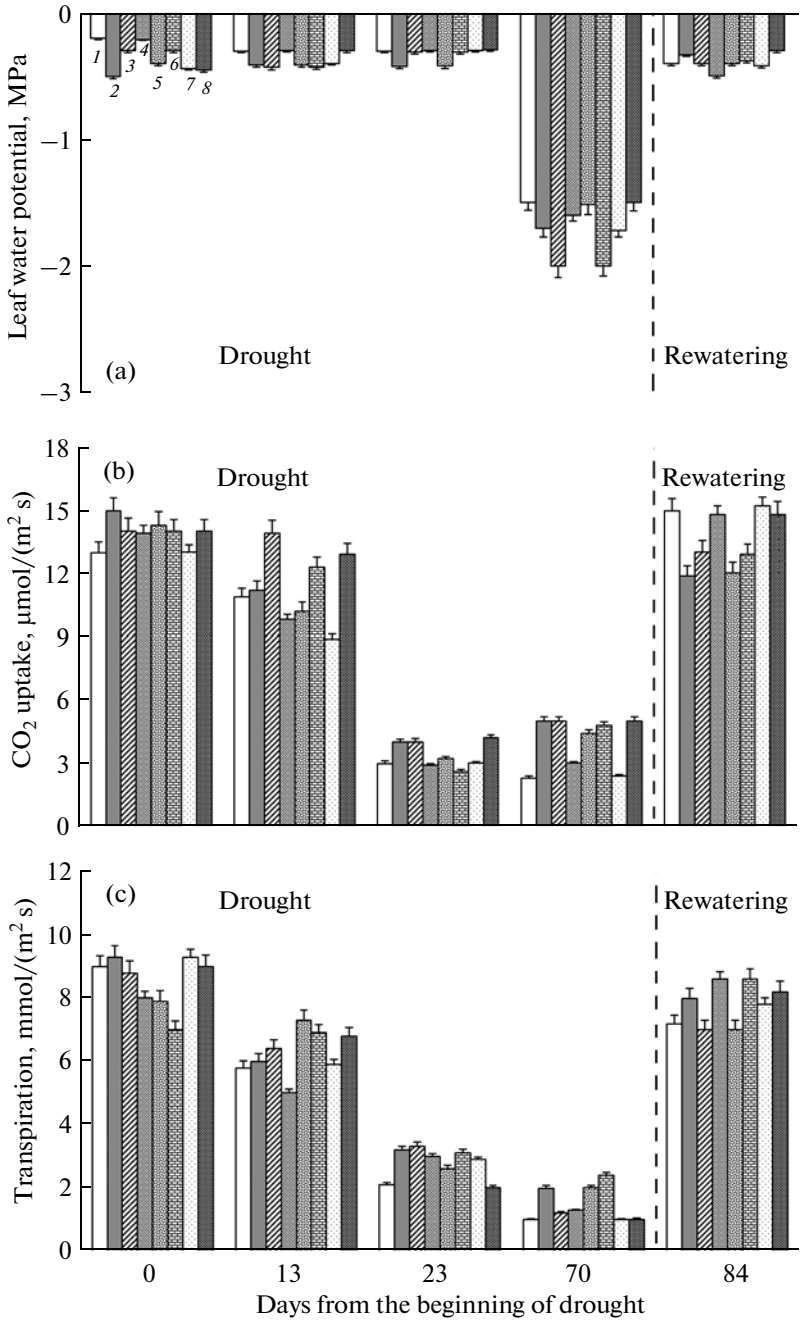

Fig. 1. Trends of leaf water potential (a), CO_2 uptake (b), and transpiration (c) in drought-stressed plants at 0, 13, 23, 70, and 84 days from the beginning of the experimental period.
(*1*) *P. communis*; (*2*) *P. eleagnifolia*; (*3*) *P. orientalis*; (*4*) *P. lycioides*; (*5*) *P. reuteri*; (*6*) *P. arabica*, (*7*) *P. glauca*; (*8*) *P. scoparia*.
Each value represents the mean of 30 measurements from three plants.

Generally, AsA and DHA content increased with prolongation of drought stress in all species examined, with a slight decrease at the highest level of water deficit for DHA (Figs. 3a, 3b). In the first 13 days of the drought phase, the ratios AsA/DHA were not significantly different from those of control plants, whereas, at the higher levels of water deficit, AsA/DHA decreased and subsequently showed an increase in the last days of the drought period (Table 1). Drought stress caused marked increases in the GSH content, with the exception of *P. lycioides* and *P. arabica* (Fig. 3c). GSSG levels showed a slight increase during the progressive water shortage, particularly marked in *P. glauca* and *P. scoparia*, followed by a decrease at the maximum level of drought stress (Fig. 3d). Generally, AsA/DHA and GSH/GSSG increased with increasing drought

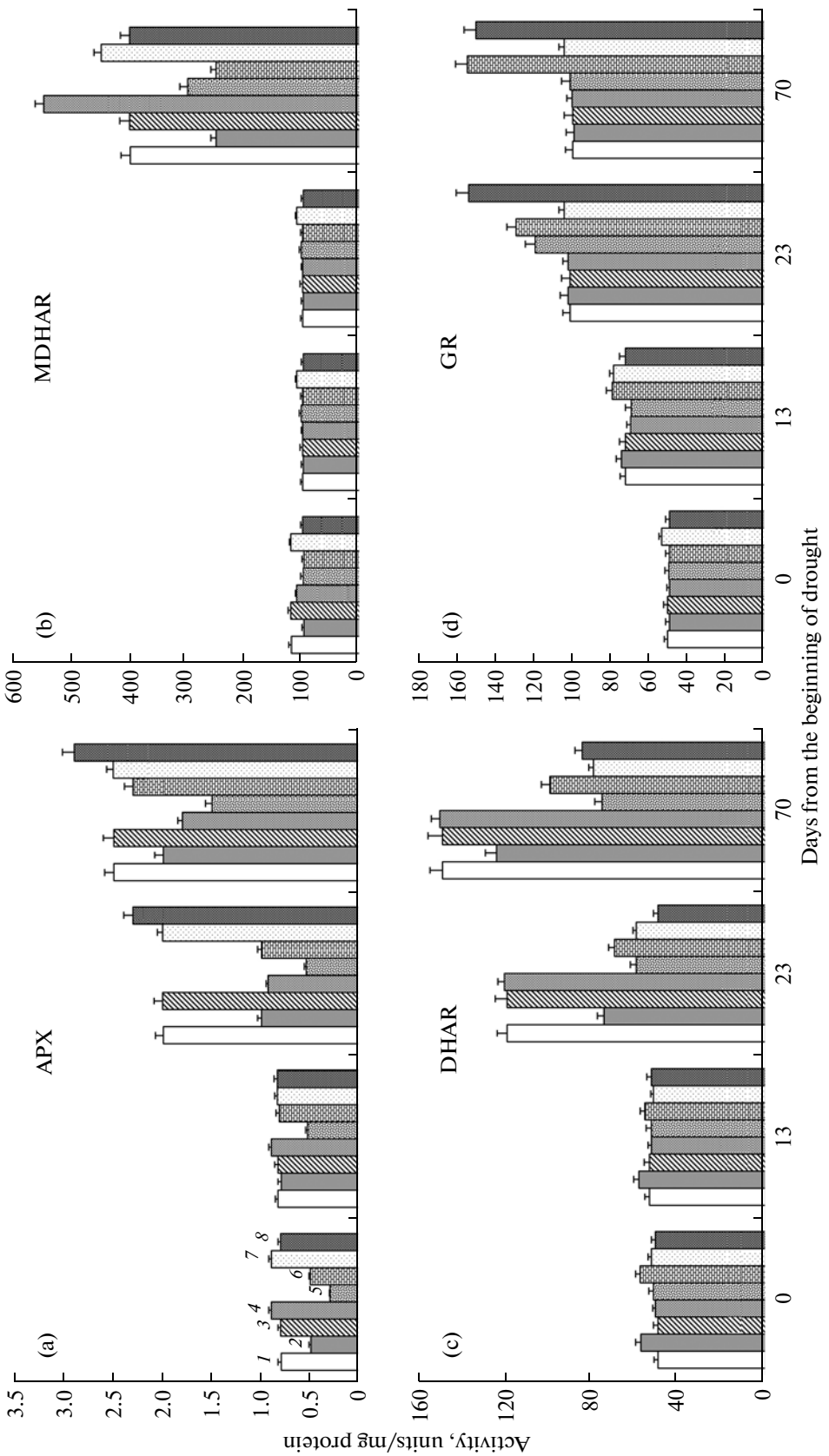

Fig. 2. Activities of ascorbate peroxidase (APX) (a), monodehydroascorbate reductase (MDHAR) (b), dehydroascroabate reductase (DHAR) (c), and glutathione reductase (GR) (d) in drought-stressed plants at 0, 13, 23, and 70 days from the beginning of the drought period. Each value represents the mean of three measurements from three plants having a similar level of drought stress.
(*1*) *P. communis*; (*2*) *P. eleagnifolia*; (*3*) *P. orientalis*; (*4*) *P. lycioides*; (*5*) *P. reuteri*; (*6*) *P. arabica*, (*7*) *P. glauca*; (*8*) *P. scoparia*.

stress (Table 1). In all species studied, the levels of H_2O_2 were directly related to the severity of drought stress, presenting a continuous increase during the progressive drought (Fig. 3e). In control plants, the activities of APX, MADHR, DHAR, and GR, the levels of AsA, DHA, GSH, and GSSG, and the ratios AsA/DHA and GSH/GSSG remained relatively stable throughout the experimental period.

Antioxidant Enzyme Activities and Ascorbate/Glutathione Pools during the Rewatering Period

Generally, the activities of APX, MDHAR, DHAR, and GR were down-regulated during the rewatering phase and their values were lower than those found in the drought-stressed plants (Fig. 4). APX activity decreased both in shaded and sun-exposed leaves during rewatering, reaching the level in the corresponding control plants (Fig. 4a). If compared to the leaves at the most severe drought stress, the activities of MDHAR and DHAR declined both in shaded and sun-exposed leaves during the rewatering period, but they remained higher than in the relevant control plants (Figs. 4b, 4c). The decrements of MDHAR and DHAR activities were more marked in shaded leaves than in those exposed to sunlight (Figs. 4b, 4c). Both in shaded and exposed leaves, the values of GR activity decreased during rewatering in all species studied (Fig. 4d).

In both shaded and sun-exposed leaves of all the species studied, AsA and DHA content decreased during rewatering as compared to the values found during the last days of the drought phase (Figs. 5a, 5b, Table 2). The rewatering treatment also caused decreases in GSH and GSSG content both in shaded and exposed leaves (Fig. 5c). If compared to drought-stressed plants, AsA/DHA and GSH/GSSG decreased both in shaded and exposed leaves (Fig. 5d). With respect to the leaves at the most severe drought stress, the levels of H_2O_2 showed a strong decrease during the rewatering phase but remained higher in exposed than in shaded plants (Fig. 5e).

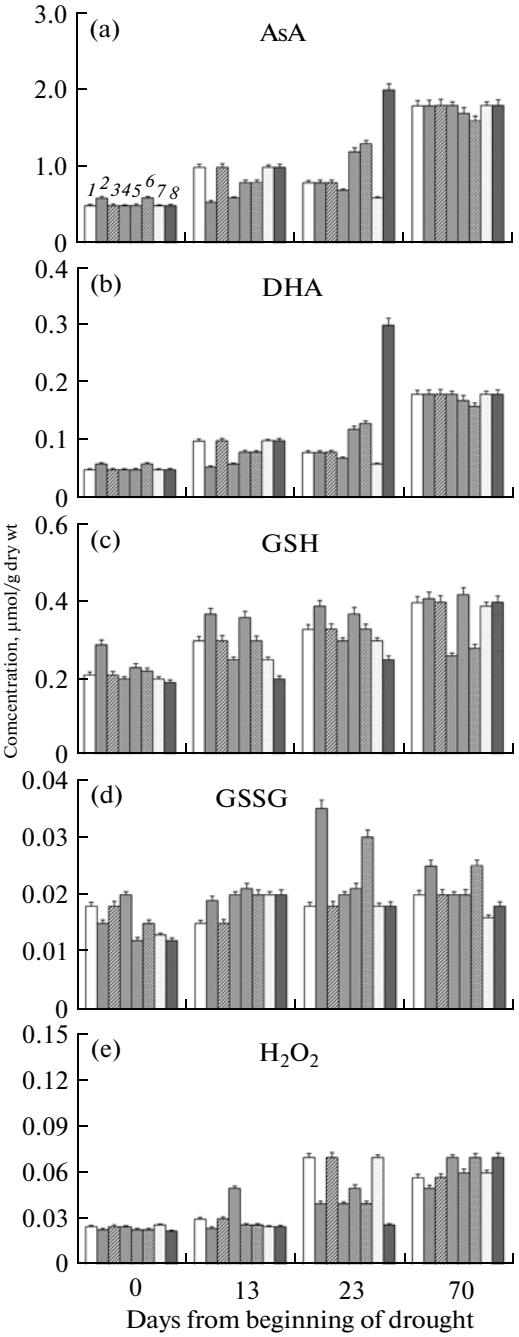

Fig. 3. Levels of ascorbate (AsA) (a), dehydroascorbate (DHA) (b), reduced glutathione (GSH) (c), oxidized glutathione (GSSG) (d), and hydrogen peroxide (e) in drought-stressed plants at 0, 13, 23, and 70 days from the beginning of the drought period. Each value represents the mean of three measurements from three plants having a similar level of drought stress.

(*1*) *P. communis*; (*2*) *P. eleagnifolia*; (*3*) *P. orientalis*; (*4*) *P. lycioides*; (*5*) *P. reuteri*; (*6*) *P. arabica*, (*7*) *P. glauca*; (*8*) *P. scoparia*.

DISCUSSION

A significant decrease in gas exchange has been observed under drought stress in species of the genus *Prunus* [10, 23]. Our results confirm that the decrease in the LWP (Fig. 2a) caused a reduction of gas exchange in *Prunus* plants (Figs. 2b, 2c). The decrease in net CO_2 assimilation in response to drought stress reduces the capacity of the photosynthetic electron transport system, which in turn causes increased levels of ROS [4]. The regulation of the activities of some antioxidant enzymes is an immediate and efficacious response to scavenge the excess of ROS, and this was observed in some fruit tree species, such as apricot [11], olive [12] and peach [13]. The results show that the activities of APX, MDHAR, DHAR, and GR and the levels of non-enzymatic antioxidants rose in all the species examined in parallel to the degree of drought,

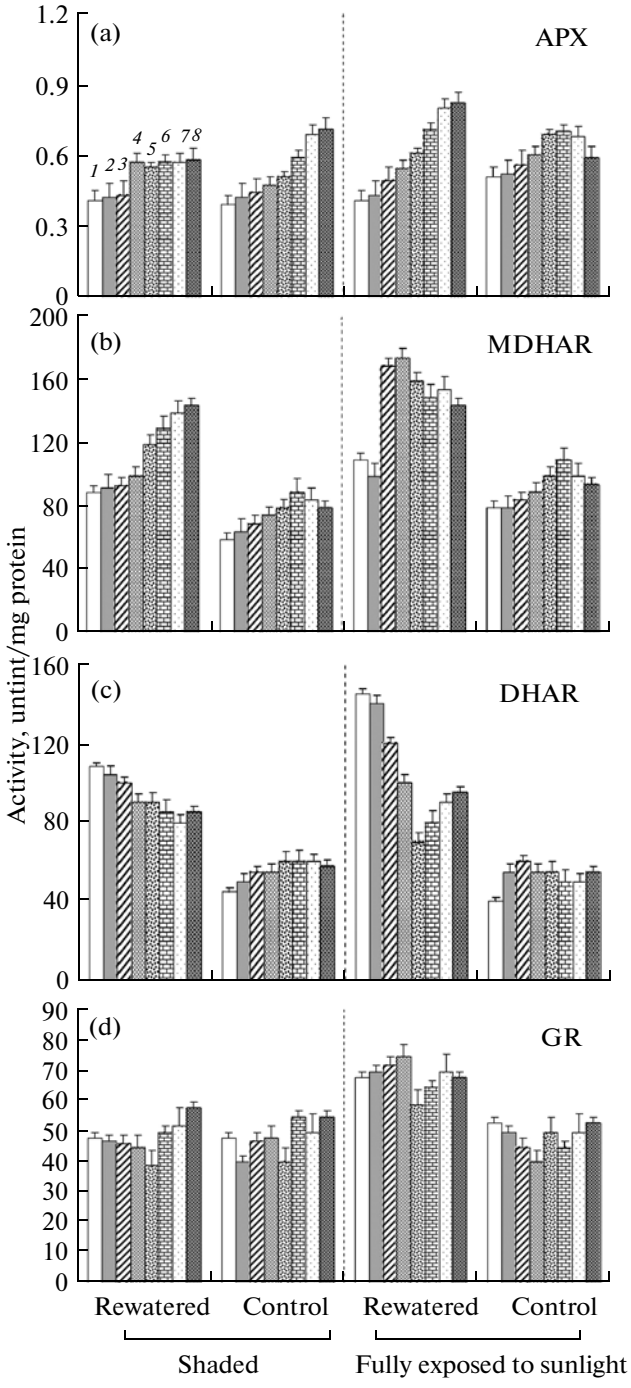

Fig. 4. Activities of ascorbate peroxidase (APX) (a), monodehydroascorbate reductase (MDHAR) (b), dehydroascorbate reductase (DHAR) (c), and glutathione reductase (GR) (d) in shaded and nonshaded leaves of rewatered plants and in the corresponding control plants. Samples were collected after 16 days from the beginning of the rewatering period. Each value represents the mean of three measurements ± SE from three plants.

(*1*) *P. communis*; (*2*) *P. eleagnifolia*; (*3*) *P. orientalis*; (*4*) *P. lycioides*; (*5*) *P. reuteri*; (*6*) *P. arabica*, (*7*) *P. glauca*; (*8*) *P. scoparia*.

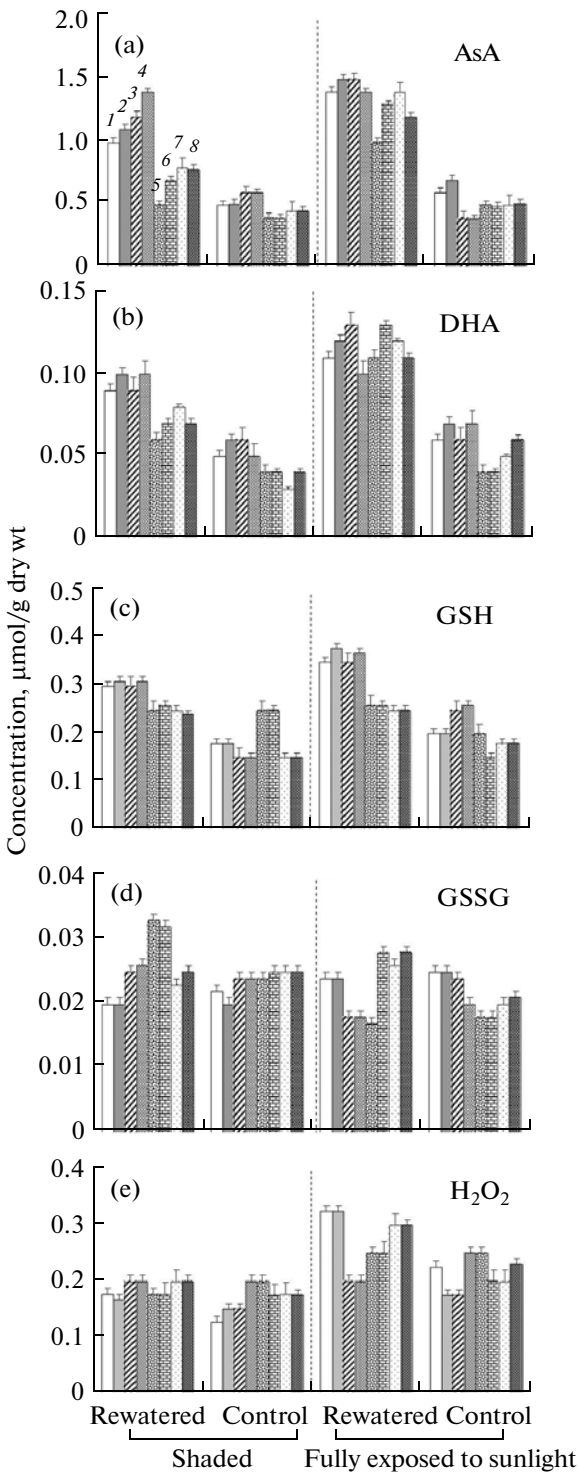

Fig. 5. Levels of ascorbate (AsA) (a), dehydroascorbate (DHA) (b), reduced glutathione (GSH) (c), oxidized glutathione (GSSG) (d), and hydrogen peroxide (e) in shaded and nonshaded leaves of rewatered plants and in the corresponding control plants. Samples were collected after 16 days from the beginning of the rewatering period. Each value represents the mean of three measurements ± SE from three plants.

(*1*) *P. communis*; (*2*) *P. eleagnifolia*; (*3*) *P. orientalis*; (*4*) *P. lycioides*; (*5*) *P. reuteri*; (*6*) *P. arabica*, (*7*) *P. glauca*; (*8*) *P. scoparia*.

with marked increases starting from 23 days from the beginning of drought, when LWP reached low values (Figs. 1a, 2, 3).

A strong linkage between APX and MDHAR trends were observed during the drought period (Fig. 3). This up-regulation reflects the strict relationship between these two enzymes. In fact, APX isozymes scavenge the H_2O_2 produced by superoxide dismutase, using AsA as the electron donor and transforming it to the free radical MDHA. Successively, MDHA can disproportionate spontaneously to AsA and DHA or be enzymatically reduced to AsA by MDHAR, a FAD enzyme which uses NADPH as a reductant [3]. During the whole period of drought stress, *P. communis, P. orientalis, P. glauca*, and *P. scoparia* showed both high APX and MDHAR activities as compared to the other species (Figs. 2a, 2b). This suggests that APX plays a key role in antioxidant protection in these three species, with a partial contribution of the other enzymes (Figs. 2, 3). Generally, the up-regulation of APX activity in all the almond species determined low levels of AsA, which is the main substrate of APX (Fig. 3a), whereas the fast turnover of MDHA, due to the high MDHAR activities, determined low levels of MDHA and, indirectly, the low amounts DHA observed (Fig. 3b).

The monomeric thiol enzyme DHAR is able to reduce DHA to AsA at expense of GSH as the electron donor, with the consequent production of GSSG, whereas GR is a flavoenzyme, which maintains the intracellular glutathione pool in the reduced state catalyzing the NADPH-dependent reduction of GSSG to GSH [6]. The significantly high values of DHAR activity found in *P. communis, P. orientalis*, and *P. lycioides* and the high activity of GR in *P. scoparia* (Figs. 2c, 2d) suggest that these almond species exert a fine regulation of glutathione homeostasis. Finally, the levels of H_2O_2 at severe drought stress were not significantly different in all the species studied (Fig. 3e). This confirms that the drought-related reduction of CO_2 assimilation (Fig. 1b) caused in all the almond species an excess of H_2O_2 deriving from the photoreduction of O_2 to $O_2^{\bullet -}$ in PSI and the following catalysis by superoxide dismutase.

The different values of AsA/DHA between almond species, starting from 23 days from the beginning of drought, reflect the changes in H_2O_2 production and conversion (Fig. 3e, Table 1). At 23 days, AsA/DHA ratios of drought-stressed plants were significantly lower than those found in control plants, in which the higher APX activity and H_2O_2 concentration were observed, with the consequent increase in DHA (Fig. 3b). The progressive loss of water from leaf tissues (Fig. 1a) caused an increase of H_2O_2-related oxidative stress (Fig. 3e), which in turn enhanced ascorbate accumulation and AsA-dependent detoxification processes (Figs. 2a, 2b). The differences in GSH/GSSG and GSH level between drought-stressed and control plants (Fig. 3c, Table 1) indicate that high levels of drought stress caused a synthesis de novo of GSH. Finally, the high activity of GR, particularly marked the last dates of the drought period, maintained the glutathione pool in the reduced status.

The recovery of water and gas exchange (Fig. 1) was accompanied by the down-regulation of the ascorbate–glutathione system of rewatered plants (Figs. 4, 5). During rewatering, the higher expression of the enzymatic antioxidant system and the higher levels of total ascorbate/glutathione pools and H_2O_2 found in exposed leaves, respectively (Figs. 4, 5), indicate that the synergic action of water deficit and high light intensity determined a higher level of oxidative stress and antioxidant protection (Figs. 4, 5). After 16 days of rewatering, H_2O_2 levels in rewatered shaded leaves were lower than those found in exposed leaves, confirming the low rate of oxidative stress in shaded leaves, also found by other authors [12, 13] in olive tree and *Prunus* rootstocks. In fruit tree species, the role of light during drought stress is of great importance. In *Prunus cerasus* L., a continuous illumination determines the photoinhibition of leaves and the irreversible damage of PSII [24]. Moreover, in other fruit species, such as olive tree, water deficit induced the "down-regulation" of PSII electron transport, and high levels of irradiance cause the higher activities of some antioxidant enzymes [12, 13].

In this study, we have found evidence for an up-regulation of ROS-scavenging enzymes and changes in ascorbate and glutathione pools as almond plants enter water deficit conditions. Furthermore, the results also confirmed that different irradiance levels, for example in different parts of the canopy, are involved in the regulation of the ascorbate–glutathione cycle under drought conditions. This response can have an important role in protecting the cellular apparatus under water deficit conditions and may be important for the selection for drought resistance in almond rootstock material. The results on antioxidant enzymes, together with those on the other compounds involved in the ascorbate–glutathione cycle, showed that the overall antioxidant protection in *P. communis, P. orientalis*, and *P. scoparia* was very strong. From an agronomic point of view, we can conclude that three almond species presented a high mean level of antioxidant response during a drought period as compared to the other almond species here studied. For this reason, they could be indicated as suitable rootstocks for almond growing in arid or semi-arid areas.

ACKNOWLEDGMENTS

The authors want to thank Shahrekord University for financial support and the Agriculture and Natural Resources Research Center of Shahrekord for collecting wild species of almond. Thanks are also to S. Mosavi for helpful information and technical assistance.

REFERENCES

1. Sathe, S.K., Wolf, W.J., Roux, K.H., Teuber, S.S., Venkatachalam, M., and Sze-Tao, K.W.C., Biochemical Characterization of Amandin, the Major Storage Protein in Almond (*Prunus dulcis* L.), *J. Agric. Food Chem.*, 2002, vol. 50, pp. 4333–4341.

2. Wijerante, S.S.K., Abou-Zaid, M.M., and Shahidi, F., Antioxidant Polyphenols in Almond and Its Co-products, *J. Agric. Food Chem.*, 2006, vol. 54, pp. 312–318.

3. Smirnoff, N., Ascorbate, Tocopherol and Carotenoids: Metabolism, Pathway Engineering and Functions, *Antioxidants and Reactive Oxygen Species in Plants*, Smirnoff, N., Ed., Oxford: Blackwell Sci., 2005, pp. 53–66.

4. Vranová, E., Inzé, D., and van Breusegem, F., Signal Transduction during Oxidative Stress, *J. Exp. Bot.,* 2002, vol. 53, pp. 1227–1236.

5. Horemans, N., Foyer, C.H., and Asard, H., Transport and Action of Ascorbate at the Plant Plasma Membrane, *Trends Plant Sci.*, 2000, vol. 5, pp. 263–267.

6. Foyer, C.H., Gomez, L.D., and van Heerdern, P.D.R., Glutathione, *Antioxidants and Reactive Oxygen Species in Plants*, Smirnoff, N., Ed., Oxford: Blackwell Sci., 2005, pp. 53–66.

7. Esparza, G., DeJong, T.M., Weinbaum, S.A., and Klein, I., Effects of Irrigation Deprivation during the Harvest Period on Yield Determinants in Mature Almond Trees, *Tree Physiol.*, 2001, vol. 21, pp. 1073–1079.

8. Klein, I., Esparza, G., Weinbaum, S.A., and DeJong, T.M., Effects of Irrigation Deprivation during the Harvest Period on Leaf Persistence and Function in Mature Almond Trees, *Tree Physiol.,* 2001, vol. 21, pp. 1063–1072.

9. Gomes-Laranjo, J., Coutinho, J.P., Galhano, V., and Cordeiro, V., Responses of Five Almond Cultivars to Irrigation: Photosynthesis and Leaf Water Potential, *Agricult. Water Management,* 2006, vol. 83, pp. 261–265.

10. Rouhi, V., Samson, R., Lemeur, R., and van Damme, P., Photosynthetic Gas Exchange Characteristics in Three Different Almond Species during Drought Stress and Subsequent Recovery, *Environ. Exp. Bot.*, 2007, vol. 59, pp. 117–129.

11. Scebba, F., Sebastiani, L., and Vitagliano, C., Activities of Antioxidant Enzymes during Senescence of *Prunus armeniaca* Leaves, *Biol. Plant.*, 2001, vol. 44, pp. 41–46.

12. Sofo, A., Dichio, B., Xiloyannis, C., and Masia, A., Effects of Different Irradiance Levels on Some Antioxidant Enzymes and on Malondialdehyde Content during Rewatering in Olive Tree, *Plant Sci.,* 2004, vol. 166, pp. 293–302.

13. Sofo, A., Tuzio, A.C., Dichio, B., and Xiloyannis, C., Influence of Water Deficit and Rewatering on the Components of the Ascorbate–Glutathione Cycle in Four Interspecific *Prunus* Hybrids*, Plant Sci.,* 2005, vol. 169, pp. 403–412.

14. Browicz, K. and Zohary, D., The Genus *Amygdalus* L. (Rosaceae): Species Relationships, Distribution and Evolution under Domestication, *Genet. Res. Crop Evol.,* 1996, vol. 43, pp. 229–247.

15. Sorkheh, K., Shiran, B., Gradziel, T.M., Epperson, B.K., Martinez-Gomez, P., and Asadi, E., Amplified Fragment Length Polymorphism as a Tool for Molecular Characterization of Almond Germplasm: Genetic Diversity among Cultivated Genotypes and Related Wild Species of Almond, and Its Relationships with Agronomic Traits, *Euphytica*, 2007, vol. 156, pp. 327–344.

16. Sorkheh, K., Shiran, B., Rouhi, V., Asadi, E., Jahanbazi, H., Moradi, H., Gradziel, T.M., and Martínez-Gómez, P., Phenotypic Diversity within Native Iranian Almond Species and Their Breeding Potential, *Genet. Res. Crop Evol.*, 2009, vol. 56, pp. 947–961.

17. Goudriaan, J. and van Laar, H.H., *Modeling Potential Crop Growth Processes,* Dordrecht: Kluwer, 1994.

18. Foyer, C.H., Rowell, J., and Walker, D.A., Measurement of the Ascorbate Content of Spinach Leaf Protoplasts and Chloroplasts during Illumination, *Planta*, 1983, vol. 157, pp. 239–244.

19. Carlberg, I. and Mannervik, B., Glutathione Reductase, *Methods Enzymol.*, 1985, vol. 113, pp. 484–490.

20. Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., and Klenk, D.C., Measurement of Protein Using Bicinchoninic Acid, *Anal. Biochem.*, 1985, vol. 150, pp. 76–85.

21. Griffith, O.W., Determination of Glutathione and Glutathione Disulfide using Glutathione Reductase and 2-Vinylpyridine, *Anal. Biochem.,* 1989, vol. 106, pp. 207–212.

22. Lee, D.H. and Lee, C.B., Chilling Stress-Induced Changes of Antioxidant Enzymes in the Leaves of Cucumber: In Gel Enzyme Activity Assays, *Plant Sci.*, 2000, vol. 159, pp. 75–85.

23. Ruiz-Sánchez, M.C., Domingo, R., Torrecillas, A., and Pérez-Pastor, A., Water Stress Preconditioning to Improve Drought Resistance in Young Apricot Plants, *Plant Sci.,* 2000, vol. 156, pp. 245–251.

24. Layne, D.R. and Flore, J.A., Physiological Response of *Prunus cerasus* to Whole-Plant Source Manipulation. Leaf Gas Exchange, Chlorophyll Fluorescence, Water Relations and Carbohydrate Concentrations, *Physiol. Plant.,* 1993, vol. 88, pp. 44–51.