Available online at www.sciencedirect.com
SCIENCE DIRECT
Plant Science 169 (2005) 403–412
PLANT SCIENCE
www.elsevier.com/locate/plantsci

Influence of water deficit and rewatering on the components of the ascorbate–glutathione cycle in four interspecific *Prunus* hybrids

Adriano Sofo *, Angelo Carmine Tuzio, Bartolomeo Dichio, Cristos Xiloyannis

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

Received 31 January 2005; received in revised form 6 April 2005; accepted 6 April 2005
Available online 25 April 2005

Abstract

The activities of 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), as well as the levels of ascorbate pool, glutathione pool and H_2O_2 were studied in plants of four interspecific *Prunus* hybrids subjected to water deficit and shade conditions. After 70 days of water shortage, plants were subjected to a rewatering treatment. During water recovery, leaves fully exposed to sunlight and leaves in shade conditions of about 30% of environmental irradiance were sampled. After 70 days without irrigation, mean pre-dawn leaf water potential of all the hybrids fell from −0.34 to −3.30 MPa and marked decreases in net photosynthesis and transpiration occurred. The activities of APX, MDHAR, DHAR and GR increased in relation to the severity of drought stress in all the clones studied. Generally, APX, MDHAR, DHAR and GR were down-regulated during the rewatering phase and their activities decreased faster in shaded leaves than in non-shaded leaves. The levels of total ascorbate, total glutathione and H_2O_2 were directly related to the increase of drought stress and subsequently decreased during rewatering. This response could limit cellular damage caused by active oxygen species during periods of water deficit. The ability of *Prunus* hybrids to regulate the enzymatic antioxidant system during different water and irradiance conditions might be an important attribute linked to drought tolerance.

Keywords: Antioxidant enzymes; Ascorbate peroxidase; Dehydroascorbate reductase; Drought stress; Glutathione reductase; Oxidative stress

1. Introduction

Acclimation of plants to drought is often associated with increased levels of activated oxygen species (AOS), such as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$) and singlet oxygen ($^1O_2$), which are toxic for the cells [1,2]. AOS are by-products of aerobic metabolism and their production is enhanced during drought conditions through the disruption of electron transport system and oxidizing metabolic activities occurring in chloroplasts, mitochondria and microbodies [3,4]. Excessive levels of AOS damage cellular structures and macromolecules, causing photoinhibition of photosynthetic apparatus [1] but the production and accumulation of AOS activate multiple defence responses, thus having also a positive role [4,5]. In particular, the presence of H_2O_2 in the apoplast is toxic for pathogens, is involved in gene transcription and systemic acquired resistance, and slows down the spread of invading organisms by cell death round the infection and a rapid local cross-linking of the cell wall [6,7].

Under non-stressful conditions, AOS are efficiently eliminated by non-enzymatic and enzymatic antioxidants, whereas during drought conditions the production of AOS exceeds the capacity of the antioxidative systems to remove them, causing oxidative stress [1,8]. The antioxidant non-

Abbreviations: AOS, activated oxygen species; APX, ascorbate peroxidase; AsA, ascorbate; CP, control plants; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; GR, glutathione reductase; GSH, glutathione; GSSG, glutathione disulfide; LWP, leaf water potential; MDHA, monodehydroascorbate; MDHAR, monodehydroascorbate reductase; NSL, non-shaded leaves; PAR, photosynthetic active radiation; SL, shaded leaves; SP, stressed plants

* Corresponding author. Tel.: +39 0971 205269; fax: +39 0971 205378.
E-mail address: sofo@unibas.it (A. Sofo).

0168-9452/$ – see front matter
doi:10.1016/j.plantsci.2005.04.004

enzymatic system includes ascorbate and glutathione, two constituents of the antioxidative ascorbate–glutathione cycle which detoxify H_2O_2 in the chloroplasts [3] and are located both within the cell and in the apoplast [6,9]. Ascorbate (AsA) is a major primary antioxidant synthesized on the inner membrane of the mitochondria which reacts chemically with $^1O_2$, $O_2^{\bullet -}$, $HO^{\bullet}$ and thiyl radical [3,8], and acts as the natural substrate of many plant peroxidases [10]. Moreover, AsA is involved in other functions such as plant growth, gene regulation, modulation of some enzymes and redox regulation of membrane-bound antioxidant compounds [6–8]. Glutathione (GSH) is a tripeptide synthesized in the cytosol and the choloroplast which scavenges $^1O_2$ and H_2O_2 and is oxidized to glutathione disulfide (GSSG) when acts as an antioxidant and redox regulator [1,8,11]. GSH is the substrate of glutathione *S*-transferases (GSTs), which have a protective role in the detoxification of xenobiotics and dehydroascorbate reductase (DHAR) [9]. Finally, GSH is a precursor of phytochelatins, which regulate cellular heavy metals levels, and is involved in gene expression [8].

The antioxidant enzymatic system includes the enzymes of the ascorbate–glutathione cycle, that operates both in the chloroplasts and in the cytosol: 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).

The genus *Prunus* comprises more than 400 species adapted to temperate areas and cultivated in Europe [12]. In particular, stone fruit crops, such as peach (*Prunus persica* L.), plum (*Prunus cerasifera* L. and *Prunus domestica* L.), almond (*Prunus dulcis* L.), apricot (*Prunus armeniaca* L.) and cherry tree (*Prunus avium* L.), 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 lack of precipitation. Productive stone fruit trees are usually grafted plants with a lower part, the rootstock and an upper grafted part, which is the genotype of the commercial variety. Rootstocks have different genetic background compared to the commercial varieties and can be used to confer various traits, such as drought stress resistance. Many plum genotypes are used as rootstock for almost all other *Prunus* species and, among them, Myrobalan plum (*P. cerasifera* L.) clones show positive agronomic features and are resistant to root-knot nematodes [12]. The response to water deficit of these species is a well documented process [13–17] but only few studies highlighted the importance of antioxidant enzymes in genus *Prunus* and in other fruit trees [18,19], and very little is known about the linkages between drought and the components of the ascorbate–glutathione cycle in these species.

The aim of this work was to study the changes of antioxidant enzyme activities (APX, MDHAR, DHAR and GR) and the level of some compounds (ascorbate and glutathione pools and H_2O_2) involved in the ascorbate–glutathione cycle in plants of four *Prunus* interspecific hybrids grown under water shortage followed by a rewatering phase, and to determine the differences of antioxidant and physiological responses among hybrids during stress conditions. Finally, on the basis of previous findings [19], we also hypothesize different patterns of enzyme activities in leaves under different levels of irradiance during the rewatering phase.

2. Materials and methods

2.1. Study site, plant material and experimental design

The study site was located at the 'Università degli Studi della Basilicata' in Potenza (Southern Italy – Basilicata Region – 40°39′N, 15°47′E). The experimental period started on July 10 and ended on October 24, 2002.

Trials were conducted on virus free plant material obtained from the breeding programs of INRA Bordeaux and SIA Zaragoza (EU funded project FAIR-6-CT-98-4139). The material, presenting different levels of resistance against nematodes of *Meloidogyne* spp., included four interspecific hybrids named 'P3605' (*Prunus amygdalus* L. 'Garfi' × *P. persica* L. 'Nemared'), '8–9' (*P. cerasifera* L. 'P2980' × 'P3605'), '7–7' (*P. cerasifera* L. 'P2175' × *Prunus davidiana* L.) and '6–5' (*P. cerasifera* L. 'P2175' × *P. amygdalus* L. 'Garfi').

The experimental scheme was carried out using 1-year-old rootstocks planted in spring 2001, 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. Trees were irrigated with drip emitters per plant discharging 3 L h^{-1}. Soil water content was maintained at a constant value of around 85% of the field water capacity by integrating the amount of water lost through transpiration during the day. Plants were fertilized at 25-day intervals throughout the period of vegetative growth with 3.5 g of slow release nitrogen complex fertilizer Nitrophoska Gold—BASf-15N-9P-15 K + 2 Ca + 17.5 Mg (Compo Agricoltura, Cesano Maderno, MI, Italy).

Plants were divided in two groups: drought-stressed plants (SP) and control plants (CP). CP were maintained in an optimal soil water conditions (85% of the field water capacity) during the whole experimental period, whereas SP subjected to a water shortage period starting from July 10 to September 18. Containers of SP were covered with plastic film in order to avoid rainfall infiltrations and evaporation from the soil surface. After this 70-day period of drought, stressed plants were subjected to a rewatering treatment of 36 days (from September 19 to October 24).

2.2. Environmental parameters, gas exchange and water status

For each day of the experimental period, measurements of air temperature and relative humidity (RH) were taken by a data logger Tinytag Ultra 2 K (Maeco, Cranleigh, Surrey,