



The application of ascorbate or its immediate precursor, galactono-1,4-lactone, does not affect the response of nitrogen-fixing pea nodules to water stress

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Summary

Nitrogen fixation in legumes is dramatically inhibited by abiotic stresses, and this reduction is often associated with oxidative damage. Although ascorbate (ASC) has been firmly associated with antioxidant defence, recent studies have suggested that the functions of ASC are related primarily to developmental processes. This study examines the hypothesis that ASC is involved in alleviating the oxidative damage to nodules caused by an increase in reactive oxygen species (ROS) under water stress. The hypothesis was tested by supplying 5 mM ASC to pea plants (*Pisum sativum* L.) experiencing moderate water stress (ca. -1 MPa) and monitoring plant responses in relation to those experiencing the same water stress without ASC. A supply of exogenous ASC increased the nodule ASC+dehydroascorbate (DHA) pool compared to water-stressed nodules without ASC, and significantly modulated the response to water stress of the unspecific guaiacol peroxidase (EC 1.11.1.7) in leaves and nodules. However, ASC supply did not produce recovery from water stress in other nodule antioxidant enzymes, nodule carbon and nitrogen enzymes, or nitrogen fixation. The supply of the immediate ASC precursor, galactono-1,4-lactone (GL), increased the nodule ASC+DHA pool, but also failed to prevent the decline of

Abbreviations: APX, ascorbate peroxidase; ASC, ascorbate; DHA, dehydroascorbate; DW, dry weight; GL, galactono-1, 4-lactone; GPX, guaiacol peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; ICDH, NADP⁺-dependent isocitrate dehydrogenase; NDW, nodule dry weight; ROS, reactive oxygen species; SOD, superoxide dismutase; SS, sucrose synthase; WS, water-stressed (plants or nodules); WSA, water-stressed (plants or nodules) supplied with 5 mM ASC; WS-GL, water-stressed (plants or nodules) supplied with 5 mM GL

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nitrogen fixation and the reduction of carbon flux in nodules. These results suggest that ASC has a limited role in preventing the negative effects of water stress on nodule metabolism and nitrogen fixation.

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Introduction

Drought has a negative impact on nitrogenase activity in legume nodules (Sprent, 1972). It remains to be elucidated, however, whether C, O or N fluxes are ultimately responsible for regulating the nitrogen fixation process under water stress (Marino et al., 2006). Down-regulation of sucrose synthase (SS; EC 2.1.4.13) activity is one of the earliest events occurring in nodules subjected to either water stress (Gálvez et al., 2005) or to a range of other environmental stresses (Arrese-Igor et al., 1999). In addition, recent studies point to an involvement of reactive oxygen species (ROS) in the signaling processes leading to SS and nitrogen fixation down-regulation under adverse conditions (Marino et al., 2006).

Aerobic organisms suffer the ubiquitous danger of ROS; this risk is especially pronounced in legume root nodules (Becana et al., 2000). When legumes are subjected to water stress, oxidative stress occurs in nodules and an antioxidant defence system is activated (Iturbe-Ormaetxe et al., 1998). However, this defence system is not able to neutralize the overproduction of ROS. Indeed, the decline of nitrogen fixation under water stress is often associated with declines in the activities of the antioxidant enzymes as well as the depletion of and (homo)glutathione pools (Gogorcena et al., 1995).

Some studies have described ASC as a plant growth modulator (Chinoy, 1984; Arrigoni and De Tullio, 2002; Potters et al., 2002) and recent studies have shown that ASC controls the expression of many development-related genes through hormone signaling (Pastori et al., 2003). However, due to its antioxidant properties (Noctor and Foyer, 1998), and its implication within the Halliwell–Asada cycle, it has been generally assumed that the main role of ASC is related to the enhancement of antioxidant capacity in response to stress. For example, Shalata and Neumann (2001) showed an increasing tolerance to salinity of salt-stressed tomato seedlings supplied with ASC, and others have suggested ASC biosynthesis manipulation as a strategy for improving abiotic stress tolerance (Guo et al., 2005). Nevertheless, the effect of ASC level modulation on plant gene expression differs significantly from that provoked by ROS modulation

and the ASC effect cannot be regarded as simply a reversal of the ROS effect (Foyer and Noctor, 2005a, b).

The beneficial effect of ASC for nitrogen fixation is revealed by nodule effectiveness being strongly correlated with high activity of the ASC–glutathione cycle (Dalton et al., 1993). Further, Bashor and Dalton (1999) showed that an external ASC supply to non-stressed soybean plants led to improved nodule functioning, which was related to striking increases in nitrogenase activity, nodule leghemoglobin content and ascorbate peroxidase (APX; EC 1.11.1.11) activity. Very recently, a strong positive correlation was observed between nitrogenase activity and nodule ASC and glutathione contents, although these authors could not find any evidence for oxidative stress to be involved in the natural senescence of pea nodules (Groten et al., 2005). Because of the apparent beneficial effects of ASC on nitrogen fixation under optimal conditions of water availability, and its potential antioxidant role, the aim of this study was to ascertain whether ASC could also play a beneficial role for nitrogen-fixing legumes during ROS overproduction under drought. This was achieved by supplying ASC, or its immediate precursor, L-galactono-1,4-lactone (GL), to nodulated pea plants experiencing water stress and monitoring physiological and water status parameters, nodule antioxidant responses and key carbon and nitrogen enzyme activities.

Materials and methods

Growth conditions

Pea plants (*Pisum sativum* L. cv. Sugar snap) were inoculated with *Rhizobium leguminosarum* biovar. *viciae* strain NLV8, a hup minus strain (Gálvez et al., 2005). Plants were grown in 1 L pots of a vermiculite/perlite mixture (1/1) (v/v) and watered with a nutrient solution lacking nitrogen (Rigaud and Puppo, 1975) in a controlled environment chamber (25/18 °C, 60/80% r.h., 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (PPF) and 15 h photoperiod).

Experimental design, water relations and gas exchange measurements

In a first set of experiments, 4-week-old pea plants were divided randomly into three sets: control plants

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