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Secondary metabolism and antioxidants are involved in environmental adaptation and stress tolerance in lettuce

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Summary

Lettuce (*Lactuca sativa*) plants grown in a protective environment, similar to *in vitro* conditions, were acclimated in a growth chamber and subjected to water stress to examine the activation of genes involved in secondary metabolism and biosynthesis of antioxidants. The expression of phenylalanine ammonia-lyase (PAL), γ-tocopherol methyl transferase (γ-TMT) and L-galactose dehydrogenase (L-GalDH) genes involved in the biosynthesis of phenolic compounds, α-tocopherol and ascorbic acid, respectively, were determined during plant adaptation. These genes were activated in tender plants, grown under protective conditions, when exposed to normal growing conditions in a growth chamber. A large increase in transcript level for PAL, a key gene in the phenylpropanoid pathway leading to the biosynthesis of a wide array of phenolics and flavonoids, was observed within 1 h of exposure of tender plants to normal growing conditions. Plant growth, especially the roots, was retarded in tender plants when exposed to normal growing conditions. Furthermore, exposure of both protected and unprotected plants to water stress resulted in the activation of PAL. PAL inhibition by 2-aminoindan-2-phosphonic acid (AIP) rendered these plants more sensitive to chilling and heat shock treatments. These results suggest that activation of secondary metabolism as well as the antioxidative metabolism is an integral part of plant adaptation to normal growing conditions in lettuce plants.
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Abbreviations: AIP, 2-aminoindan-2-phosphonic acid; L-GalDH, L-galactose dehydrogenase; P, protected plants; PAL, phenylalanine ammonia-lyase; PPFD, photosynthetic photon flux density; PWS, protected plants under water stress; RH, relative humidity; γ-TMT, γ-tocopherol methyl transferase; U, unprotected plants; UWS, unprotected plants under water stress.

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Introduction

Plants grown *in vitro* or in protective environments lack necessary adaptive mechanisms to thrive under harsher environmental conditions typically found in a growth chamber, greenhouse or field (Van Huylenbroeck and Debergh, 1996). These plants, therefore, need to acclimate before they can be successfully grown under *ex vitro* conditions which involve unregulated and often highly fluctuating environmental conditions (Pospíšilová et al., 1999; Van Huylenbroeck et al., 2000; Ziv, 1986). Tender plants grown *in vitro* without acclimation often fail to establish, and perform poorly in *ex vitro* conditions. Indeed, it is a common practice to acclimate *in vitro* propagated or protectively cultivated plants by gradual exposure to harsher environmental conditions to ensure their successful establishment and growth (Bañon et al., 2006; Dhawan and Bhojwani, 1987). Despite the recognition that these plants need to undergo an adaptive process to grow successfully under normal growing conditions, very little is known about the nature of such adaptation. Although plant responses and adaptations to various environmental stresses have been studied extensively (Guy, 1990; Levitt, 1980), they have not been examined in relation to environmental fluctuations typically found in normal growing conditions.

There is overwhelming evidence that many antioxidants play a key role in plant adaptation to both abiotic and biotic stresses (Burritt and MacKenzie, 2003; Dixon and Paiva, 1995; Vranová et al., 2002). Plants typically produce a diverse group of antioxidants as a protective mechanism against oxidative compounds which are produced in response to various stresses and known to have a damaging effect on membranes, organelles and macromolecules (Mittler, 2002; Noctor and Foyer, 1998; Smirnoff, 1998). A significant part of antioxidants produced by plants in response to stress is secondary metabolites, including a vast array of simple and complex phenolic compounds derived primarily via the phenylpropanoid pathway (Dixon and Paiva, 1995). The two aromatic amino acids, phenylalanine and tyrosine, which are the products of the shikimate pathway, play an important role in this regard as the former is a precursor for a wide variety of phenolics while the latter a precursor for tocopherols. A well-characterized and a key gateway enzyme in the phenylpropanoid pathway is phenylalanine ammonia-lyase (PAL; EC 4.3.1.5). It has been well documented that the gene encoding this protein is responsive to a number of abiotic and biotic stresses in many plant species (Diallinas and Kanellis, 1994; Liu et al., 2006; Reymond et al., 2000). In response to environmental stresses, an increase in PAL activity and an activation of a number of genes involved in phenylpropanoid pathway have been shown in many plant species (Keles and Oncel, 2002; Leyva et al., 1995).

In addition, other important antioxidants such as tocopherols and ascorbic acid have been shown to be involved in plant adaptation to various stresses. Tocopherols are strong antioxidants which respond to various stresses and have been shown to protect plants against oxidative species, stabilize membranes (Havaux et al., 2003) and participate in intracellular signaling (Munné-Bosch, 2005). L-ascorbic acid is a commonly occurring major antioxidant in plants (Noctor and Foyer, 1998; Smirnoff et al., 2001) and is responsive to various stresses (Smirnoff and Wheeler, 2000). Hence it is thought to play an important role in plant adaptation (Conklin et al., 1996; Shalata and Neumann, 2001). Two enzymes, γ-tocopherol methyl transferase (γ-TMT; EC 2.1.1.95) and L-galactose dehydrogenase (L-GalDH; EC 1.1.1.117), play an important role in the biosynthesis of α-tocopherol and ascorbic acid, respectively. γ-TMT participates in the last rate-limiting step in the biosynthesis of α-tocopherol by methylation of γ-tocopherol (Bergmüller et al., 2003; Shintani and DellaPenna, 1998). L-GalDH is a key regulating enzyme involved in the oxidation of galactose to L-galactono-1,4-lactone leading to the formation of ascorbic acid (Gatzek et al., 2002; Wheeler et al., 1998). Although a number of these antioxidants have been known to be involved in plant adaptation to various biotic and abiotic stresses, their role in plant function under non-stressful natural environmental conditions is not known.

The primary objective of this study is to determine the role of antioxidants, including some that are the products of secondary metabolism, in adaptation of lettuce to unprotective environmental conditions and to environmental stresses. We have examined plant responses and the activation of PAL, γ-TMT and L-GalDH genes in response to unprotective environmental conditions and environmental stresses.

Materials and methods

Plant material and growth conditions

Lettuce plants (*Lactuca sativa* L., cv. Baronet) were grown under protective conditions similar to *in vitro* culture (referred to here as tender) with relative humidity (RH) maintained around 100% under autotrophic