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Modulation of chlorogenic acid biosynthesis in *Solanum lycopersicum*; consequences for phenolic accumulation and UV-tolerance

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ABSTRACT

Chlorogenic acid (CGA) is one of the most abundant phenolic compounds in tomato (*Solanum lycopersicum*). Hydroxycinnamoyl CoA quinate transferase (HQT) is the key enzyme catalysing CGA biosynthesis in tomato. We have studied the relationship between phenolic accumulation and UV-susceptibility in transgenic tomato plants with altered HQT expression. Overall, increased CGA accumulation was associated with increased UV-protection. However, the genetic manipulation of HQT expression also resulted in more complex alterations in the profiles of phenolics. Levels of rutin were relatively high in both HQT gene-silenced and HQT-overexpressing plants raised in plant growth tunnels. This suggests plasticity in the flux along different branches of phenylpropanoid metabolism and the existence of regulatory mechanisms that direct the flow of phenolic precursors in response to both metabolic parameters and environmental conditions. These changes in composition of the phenolic pool affected the relative levels of UV-tolerance. We conclude that the capability of the phenolic compounds to protect against potentially harmful UV radiation is determined both by the total levels of phenolics that accumulate in leaves as well as by the specific composition of the phenolic profile.

1. Introduction

Phenylpropanoid-derived compounds are ubiquitous plant secondary products (Albrecht et al., 1999; Winkel-Shirley, 2002; Grotewold, 2006). These compounds are involved in a broad range of physiological and ecological processes, ranging from recruitment of pollinators and seed dispersers, interactions with pathogens, nitrogen-fixing bacteria and parasitic roots in the rhizosphere, control of male fertility, UV-tolerance, antioxidant-based defence, auxin transport, and defence against microbes and grazers (Albrecht et al., 1999; Winkel-Shirley, 2002; Grotewold, 2006). This vast range of biological functions is matched by an equally vast structural diversity. It has been estimated that some 8000 different phenolic compounds are synthesised by plants (Albrecht et al., 1999), via a variety of polymerisation, hydroxylation, methylation, glycosylation, acylation, prenylation and condensation reactions (Pourcel et al., 2007).

The accumulation of phenolic compounds is a carefully controlled process with both the levels of phenolics and the composition of the phenolic pool varying considerably between organisms, tissues, developmental stages and in relation to environmental conditions (Winkel-Shirley, 2002). Expression levels of several genes encoding key enzymes in the early part of the biosynthetic pathway, called the general phenylpropanoid pathway, are elevated in response to UV-B radiation as well as a range of other environmental signals (Weisshaar and Jenkins, 1998; Long and Jenkins, 1998). These enzymes include phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H) and 4-coumarate:coenzyme A ligase (4CL). PAL catalyses the conversion of phenylalanine to cinnamic acid, the first step of the general phenylpropanoid pathway, and this enzyme in particular controls the flux of precursors in to the phenol network and, as such, is critical in determining overall levels of phenolics that accumulate (Bate et al., 1994; Howles et al., 1996). Subsequent targeting of the phenolic precursors towards specific branches of the phenylpropanoid network is also controlled by biochemical, genetic, environmental and developmental parameters (Winkel-Shirley, 2002), and this involves the activity of a range of regulatory genes (Koes et al., 2005).

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Environmental factors like UV-B also impact on the activity of enzymes of the phenylpropanoid metabolism. For example, Wilson et al. (2001) showed that UV-B stimulates the hydroxylation reaction that converts quercetin in to kaempferol. It is likely that the allocation of phenolic substrates to individual branches of the phenylpropanoid pathway is also subject to metabolic regulation, including substrate and product feedback-loops. For example, in *Arabidopsis thaliana*, the *tt4* mutant lacks chalcone synthase (CHS) activity and consequently does not accumulate flavonoids. However, this mutant accumulates 30–60% more sinapoyl esters and this has been speculated to reflect shunting of 4-coumaroyl-coenzyme A from the blocked flavonoid pathway into sinapate biosynthesis (Li et al., 1993). The *Arabidopsis thaliana tt5* mutant also lacks flavonoids, and this is the result of impaired chalcone–flavanone isomerase activity (Li et al., 1993). However, the *tt5* mutant contains lowered levels of sinapoyl esters, and consequently, is considerably more UV-sensitive than the *tt4* mutant (Li et al., 1993; Booij-James et al., 2000). The lack of sinapoyl ester accumulation in the *tt5* mutant reflects regulatory interactions between flavonoid and sinapoyl ester biosynthesis (Li et al., 1993). In recent years our understanding of such regulatory interactions between the different branches of phenylpropanoid metabolism has been improved with the discovery of the R2R3 family of MYB transcriptional activators (Sablowski et al., 1994; Mathews et al., 2003; Verdonk et al., 2005) and repressors (Jin et al., 2000). Transcriptional de-repression, resulting from decreased expression of transcriptional repressors, appears to be one of the key mechanisms influencing flux through specific branches of the phenylpropanoid network, in response to UV-B light (Jin et al., 2000).

Phenolic compounds play a key role in protecting plants from potentially harmful UV-B radiation. In plants, UV-B has the potential to affect metabolic processes such as growth, morphogenesis, photosynthesis, and flowering. UV-B also catalyses the dimerisation of nucleotide bases which, in turn, has consequences for DNA transcription and replication (Hollosy, 2002; Jansen et al., 1998; Jordan, 1996). Numerous studies have demonstrated that UV exposure results in increases in the overall levels of phenolic compounds in response to UV exposure (Hollosy, 2002; Jansen et al., 1998; Jordan, 1996). Phenolics protect plants by specifically absorbing light of wavelengths between 280 and 340 nm, and therefore do not decrease the penetration of photosynthetically active radiation into the leaf, while filtering out harmful UV-B. Many phenolics are also effective antioxidants and this underpins the functional role of these compounds in a broad spectrum of plant stress responses. Substantial levels of phenolics accumulate in the upper epidermal cell layer, where they are mostly located in the vacuoles. As a result, little UV-B radiation (<10%) actually penetrates the mesophyll (Turunen et al., 1999), and the direct, growth-inhibiting effects of natural doses of UV-B are generally minor.

Different plant species accumulate different phenolics. The most abundant soluble phenolic in tomato leaves is chlorogenic acid (Niggeweg et al., 2004). The synthesis of CGA from quinic acid and caffeoyl CoA is catalysed by the enzyme hydroxycinnamoyl CoA quinate transferase (HQT; Niggeweg et al., 2004). We have investigated the relationship between accumulation of CGA, and other phenolic compounds, and UV-susceptibility in tomato plants (*Solanum lycopersicum* cv. Moneymaker) with altered HQT expression.

2. Results

Hydroxycinnamoyl CoA quinate transferase (HQT) is the key enzyme catalysing CGA biosynthesis in tomato. Transgenic tomato plants (*S. lycopersicum* cv. Moneymaker) that overexpress HQT (OE) or that are HQT gene-silenced (GS) have been generated and characterised by Northern blotting, as previously detailed (Niggeweg et al., 2004). Cuttings from original transformants were used in this study. Plants that were raised in growth rooms (indoors) were exposed to relatively low fluence rates of photosynthetically active radiation (PAR), and spectra that contained some UV-A (shortest wavelength 375 nm), but lacked UV-B (Fig. 1). Plants that were raised in a tunnel were exposed to relatively high, ambient light conditions, including natural background levels of UV-A and UV-B (Fig. 1). Indoor and tunnel-growth conditions differed also in respect to temperature profiles, relative humidity, and air circulation (wind).

We measured the accumulation of soluble phenolic compounds in methanol extracts. Overall, we found that the levels of soluble phenolics were relatively low in leaves of plants that were raised under growth room conditions compared to tunnel-grown plants (Fig. 2). Yet, there were significant differences between the four tomato lines indicating that alterations in HQT expression affected the overall accumulation of phenolic compounds under growth room conditions. Levels of UV absorbance were particularly low in gene-silenced plants, but significantly higher in WT plants raised in growth rooms (Fig. 2). The highest level of phenolic compounds was found in HQT-overexpressing plants. Tomato plants grown in a tunnel under natural light conditions were characterised by a major increase (up to 3.5-fold) in the overall level of UV-absorbing compounds compared to growth room-grown plants (Fig. 2). The difference between tunnel-grown and growth room-grown plants was particularly strong for the gene-silenced plants, whereas it was relatively small for the HQT-overexpressing plants. As a consequence, the relative differences in total phenolic accumulation between the four tomato lines were less pronounced under natural light conditions. We found that the HQT-overexpressing plants contained on average, some 55% more UV-absorbing pigments than gene-silenced plants under tunnel light conditions.

Chlorogenic acid is the most abundant phenolic compound in tomato leaves (Niggeweg et al., 2004). In order to determine whether altered HQT expression impacted the levels of CGA and other phenolic compounds under the growth conditions we used, we analysed the composition of the soluble phenolic pool. Methanolic extracts of leaves of tomato plants contained several UV-absorbing compounds. Peaks in the UV chromatogram were identified using mass spectrometry. The major peaks in the LC–MS spectrum represented CGA (peaks B, D, E and G) (Fig. 3). The identity of these peaks was confirmed using mass spectrometry (Fig. 4). Similar mass spectra are expected for different chlorogenic acid isomers, several of which are known to be present in tomato including 3-caffeoyl quinate (neochlorogenic acid), 4-caffeoyl quinate (cryptochlorogenic acid) and 5-caffeoyl quinate (Niggeweg et al., 2004). Also present in the spectrum were rutin (peak J) and rutin–pentose (peak I), quercetin–glucose (peak K), kaempferol–rhamnose–glucose (peak L), tomatine (peak N) and dehydrotomatine (peak M). CGA made up some 67% of total, soluble UV-absorbing material of wild type leaves raised under growth room conditions. In comparison, rutin (peaks I and J) the second most abundant phenolic in tomato leaves comprised less than 13% of total UV-absorbing capacity (Table 1). The growth-room-raised, gene-silenced lines accumulated nearly 70% less CGA than wild type plants and this was reflected in a drop in total soluble phenolics (Fig. 2). HQT-overexpressing lines, raised under growth room conditions, contained substantial amounts of CGA which comprised, on average, some 80% of the absorbance present in methanolic extracts. Compared to the wild type, HQT-overexpressing plants accumulated nearly 2.5-fold more CGA. Rutin and rutin–pentose (peaks I and J), flavonoids (peaks K and L) and tomatine levels (peaks M and N) varied little between the growth room-grown lines (Table 1). Thus, the shift in phenolic profiles