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Article Addendum

Fine-tuning regulation of strigolactone biosynthesis under phosphate starvation

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Strigolactones are signalling molecules playing a double role in the rhizosphere as host detection signals for arbuscular mycorrhizal (AM) fungi and root parasitic plants. They are biosynthetically originating from carotenoids. The biosynthesis of these signalling compounds is tightly regulated by environmental conditions such as nutrient availability, mainly phosphate (Pi). However, although it is known that limited-Pi conditions improve the production and/or exudation of strigolactones, there is no information concerning the effect of these conditions on the enzymes involved in strigolactone production. We have recently demonstrated that tomato is a good system to study the production and regulation of these important signalling compounds.[1] In the present paper we describe an analysis of Pi starvation-induced changes in gene expression in tomato roots using a microarray study. The possible role of the upregulated genes in the biosynthesis of strigolactones and their relationship with carotenoids and the hormone abscisic acid (ABA) are discussed.

In response to environmental stress conditions plants produce secondary metabolites that act as signalling molecules. For example, plants grown under limited-phosphate (Pi) conditions secrete more hyphal branching factors for arbuscular mycorrhizal (AM) fungi into the rhizosphere.[2-4] It has been demonstrated that under these conditions the exudation of strigolactones—rhizosphere signalling compounds that mediate host finding in AM fungi and root parasitic plants[2,5,6]—in red clover is significantly stimulated,[7] suggesting that Pi availability is regulating their production and/or exudation. About ten different strigolactones have so far been isolated from a wide variety of plants species all having a similar chemical structure, suggesting they are all derived from the same biosynthetic pathway. Indeed, we have demonstrated that the ABC-part of the strigolactones is derived from carotenoids, probably through oxidative cleavage by carotenoid cleavage dioxygenase (CCD) or 9-*cis* epoxycarotenoid dioxygenase (NCED) enzymes. In addition, we have postulated how, after carotenoid cleavage, further enzymatic conversions likely lead to the production of all the strigolactones known to date.[8,9] However, so far the enzymes involved in strigolactone biosynthesis and their regulation remain unknown.

We have recently demonstrated that also in tomato the biosynthesis of strigolactones is strongly promoted by Pi starvation.[1] Hence, we use tomato to elucidate the regulation of strigolactone biosynthesis. Hereto, we have analysed gene expression in response to short-, medium- and long-term Pi deprivation using the Affymetrix Tomato Genome GeneChip Array containing over 9200 genes. Analysis of transcriptome changes during short-term Pi deprivation (6 h) revealed that 230 genes were ≥2-fold upregulated. During medium-term Pi deprivation (24 h) the number of upregulated genes was reduced to 131, of which only 15 were also upregulated at 6 h (Fig. 1). This rapid but transient change in gene expression during short periods of nutrient deprivation has been reported before and was suggested to represent a non-specific stress response.[10-13] Long-term Pi deprivation (96 h) resulted in an increased number of genes induced (240 genes) of which 23 showed overlapping expression with medium-term Pi starvation (Fig. 1). Genes upregulated after prolonged Pi deprivation have been shown to be more specific to the stress applied as they are involved in improving the acquisition or use of Pi by the plant.[10,12]

Overall, the upregulated genes included genes associated with defense or stress conditions (*c.* 25%), genes related to primary metabolism (*c.* 20%), transcription factors (*c.* 7%) and genes associated with secondary metabolism (*c.* 5%). About 26% had no or insignificant homology to any known genes. More specifically, at all three time points we detected enhanced expression of genes encoding for a Pi transporter (*LePT2*), acid and purple phosphatases and kinases as well as other marker genes for Pi starvation like the iron deficiency specific-4 (*IDS4*) gene and the tomato phosphate starvation-induced (*TPSI1*) gene, which are highly indicative of a Pi starvation response.[12,14-17]

Strigolactones from maize, sorghum and cowpea are derived from the carotenoids[8] and we have recently confirmed that this is also true for tomato.[1] In spite of this carotenoid origin, the carotenoids neoxanthin, violoxanthin, β-carotene and lutein were not induced

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in the roots of tomato after Pi deprivation.[1] Indeed, genes related to the biosynthesis of carotenoids were also not affected by Pi deprivation in any of the time points and this agrees with results for other plant species exposed to Pi limiting conditions.[10-13] Strigolactones are produced in extremely low quantities[18] and therefore the amount of carotenoids already present in roots may be enough to cope with an increase in strigolactone production. Strigolactones are derived from carotenoids probably through the action of a CCD or NCED[8] (Fig. 2). Moreover, we have shown that the maize mutant *vp14*, mutated in an *NCED* gene,[19] induced less germination of the seeds of the parasitic plant *Striga hermonthica*.[8] We have also demonstrated that the tomato mutant *notabilis*, with a null mutation in the gene *LeNCED1*,[20] produces *c.* 40% less strigolactones, suggesting that the enzyme NCED1 is involved in the biosynthesis of strigolactones. However, we did not observe any increase in expression of *LeNCED1* under Pi starvation. This could imply that NCED1 is not a regulatory step in the biosynthesis of strigolactones or that the enzyme is regulated post-transcriptionally. NCEDs are known to be involved in the biosynthesis of the hormone abscisic acid (ABA) and indeed *notabilis* contains *c.* 40% less ABA than the corresponding wild-type.[20] The reduced production of strigolactones by this mutant may therefore also be due to the reduced ABA content (Fig. 2). If ABA is indeed involved in the regulation of strigolactone biosynthesis, Pi starvation may possibly exert its influence on strigolactone production through ABA. However, so far involvement of the hormone ABA in the response to Pi starvation has not been reported.[21] On the other hand, the genome of tomato is not completely sequenced and the tomato microarray contains only *c.* 26% of the tomato genes. In Arabidopsis and rice 5 different *NCEDs* have been reported.[2,22] Therefore, we cannot discard that Pi starvation affects the expression of other *NCEDs* and hence strigolactone or ABA biosynthesis.

The other class of carotenoid cleavage enzymes that has been hypothesized to be involved in the biosynthesis of strigolactones is the CCD family[2,8,22] (Fig. 2). CCDs are involved in the formation of different apocarotenoid compounds such as flavour volatiles, cyclohexenone derivatives and mycorradicin—the yellow pigments increased in the host roots upon AM colonization—and the recently reported hormone shoot multiplication signal (SMS), which is involved in shoot branching inhibition.[23-25] On the Affymetrix tomato chip only CCD1-A and CCD1-B are represented, which so far are the only *CCD* genes characterized in tomato. These genes are involved in the production of the flavour volatiles β-ionone, pseudo-ionone and geranlyacetone.[23] As for *LeNCED1*, the expression of *CCD1-A and -B* was not affected by Pi deprivation, suggesting that CCD1s are not involved in the production of strigolactones. However, as for the NCEDs, we cannot exclude that one or more of the uncharacterized tomato CCDs is/are involved in the biosynthesis of the strigolactones.

In summary, the results presented here suggest that the conversion of carotenoids to strigolactones is tightly regulated. More detailed studies will be required to find and characterise the enzymes and the transcription factors involved in strigolactone production as well as elucidate how their activity is regulated by Pi availability.

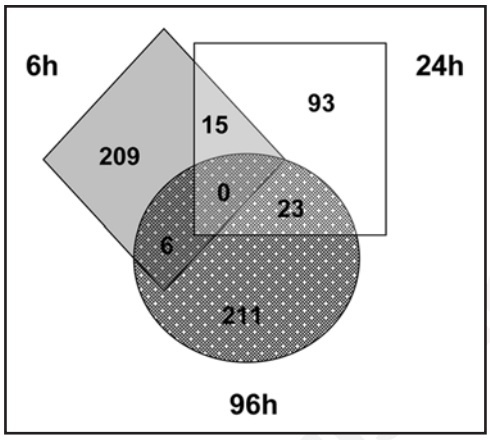

Figure 1. Microarray analysis of the temporally regulated Pi-responsive genes in tomato roots. Numbers of genes induced ≥2-fold under phosphate limiting conditions. Comparison of short-term (6 h), medium-term (24 h) and long-term (96 h) Pi starvation.

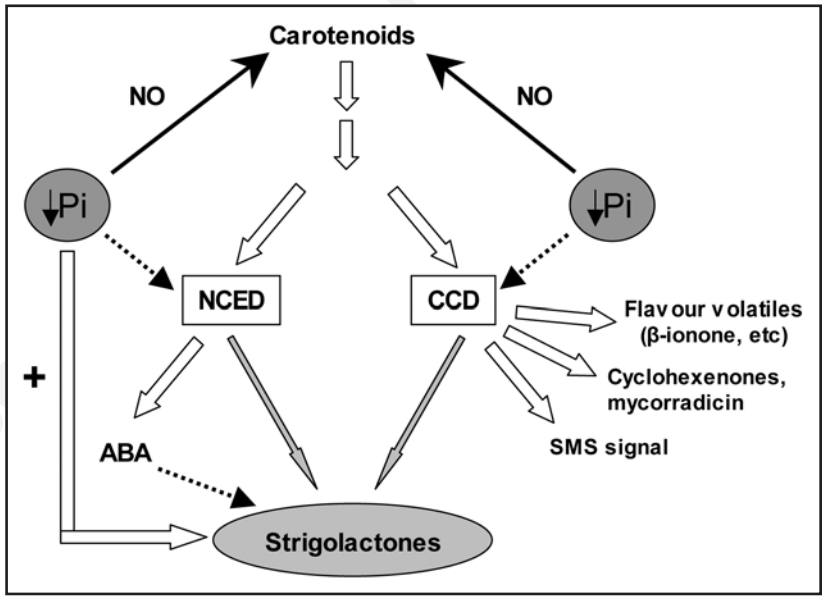

Figure 2. Schematic representation of the biosynthesis of strigolactones in plants. The known biosynthetic pathway for carotenoid-derived signalling molecules is shown in white arrows, the proposed pathways for the production of strigolactones in grey arrows. Dotted arrows indicate possible interaction between different components in the scheme. Enzymes: NCED, 9-*cis* epoxycarotenoid dioxygenase; CCD, carotenoid cleavage dioxygenase. Hormones: ABA, abscisic acid; SMS, shoot multiplication signal. NO indicates no effect.

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