

Regulation of carotenoid biosynthesis genes during plant development

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Abstract. Expression of the carotenoid biosynthesis genes *PDS* and *PSY* during development of tomato plants was studied by reverse transcriptase-polymerase chain reaction (RT-PCR). Results indicate that both stress and developmental signals control the expression of these genes. The *PDS* was mapped to the chromosome 3 of tomato.

INTRODUCTION

Plant genes for enzymes of the carotenoid biosynthesis pathway are nuclear, and the protein products are imported into organelles. Molecular probes for carotenoid biosynthesis genes are now becoming available. In the last year *PDS*, encoding phytoene desaturase, has been cloned from soybean (ref. 1), pepper (ref. 2), and tomato (G. E. Bartley and P. A. Scolnik, unpublished, Genbank accession number M88683; Pecker et al., 1992), and *PSY*, encoding phytoene synthase, was identified as corresponding to pTOM5, a cDNA for a ripening-induced tomato gene (Refs. 4, 5).

GENE EXPRESSION

We are using tomato as a model system to study the developmental aspects of carotenoid biosynthesis in plants. The low level of expression of *PDS* and *PSY* precluded studies in organs other than fruits. We have now used a sensitive mRNA detection method to study the expression of *PSY* and *PDS* during the development of tomato plants. The reverse transcriptase-polymerase chain reaction (RT-PCR) amplification assay provides the level of sensitivity required to study rare transcripts (Fig. 1). To study *PSY* and *PDS* expression by RT-PCR, we used reverse transcriptase and RNA isolated from different tomato organs to produce first-strand cDNA. We then amplified the *PDS* and *PSY* cDNAs with *Tag* polymerase, and we measured the products by ethidium bromide-induced fluorescence. Using RT-PCR we were able to detect expression of both genes in all organs of mature tomato plants. The signals from the corresponding transcripts vary significantly in the different samples, indicating control of gene expression by light and development.

The levels of *PDS* and *PSY* transcripts were determined in etiolated seedlings exposed to light and in light-grown seedlings transferred to darkness. Results indicate that *PDS* mRNA levels vary less than 2-fold during either de-etiolation or dark adaptation. *PSY* mRNA

levels also remain essentially constant during de-etiolation, but they decrease about 7-fold upon dark adaptation (Fig. 2). Thus, expression of *PDS* and *PSY* is essentially constitutive during photomorphogenesis. After photomorphogenesis is complete, the only gene which seems to be under light control is *PSY*. Colored carotenoids are required for chloroplast differentiation. Therefore, a block in their synthesis results in arrested chloroplast development, which in turn results in lower mRNA levels for several nuclear genes that encode chloroplast proteins (reviewed in ref.6).

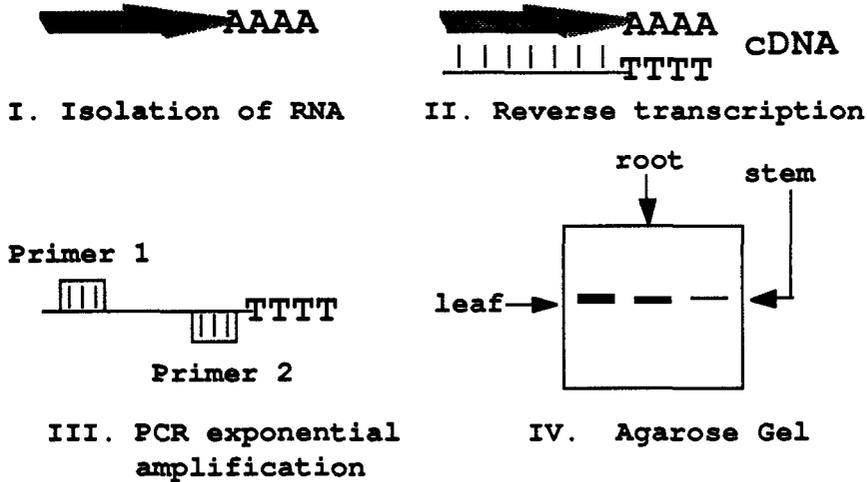


Fig. 1. Reverse transcriptase-polymerase chain reaction (RT-PCR): sensitive detection of rare mRNAs.

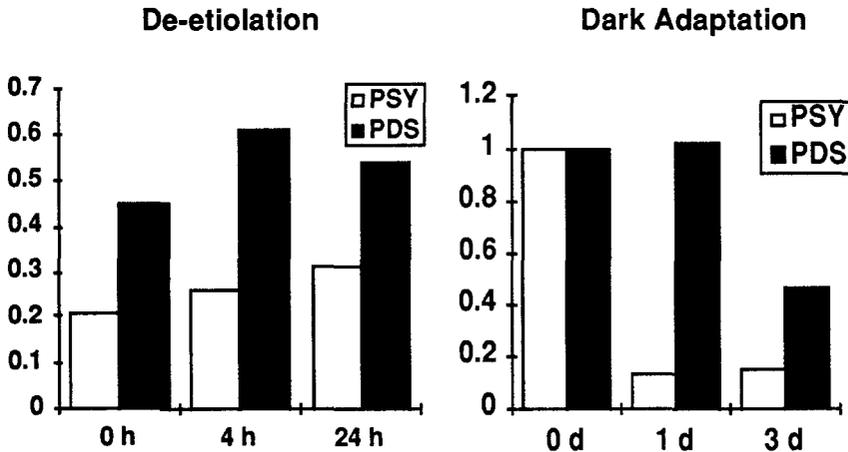


Fig. 2. Light regulation of *PSY* and *PDS* expression.

Since carotenoid genes are nuclear genes encoding chloroplast proteins, their expression was investigated in seedlings of the *ghost* mutant, which blocks the desaturation of phytoene, or wild-type seedlings treated with the Pds inhibitor Norflurazon. Seedlings that lack colored carotenoids contain increased mRNA levels for *PSY* (2-3 fold) and *PDS* (5-10 fold) (Fig. 3).

Thus, blocks in carotenoid biosynthesis induced by Norflurazon treatment or by the *ghost* mutation, induce *PDS* expression and, to a lesser extent, *PSY* expression. This induction could be due either to photooxidative stress or, as in fungi (Ref. 7), to feedback regulation of carotenogenesis. Current unpublished experiments indicate that *PDS* mRNA induction requires both an arrest in carotenoid biosynthesis and light, suggesting that this gene responds to photo-oxidation.

PSY and *PDS* expression were analyzed during leaf, flower, and fruit development. Between leaf stage 1 (approximate length 35 mm) and stage 4 (approximate length 140 mm), *PSY* mRNA levels decline slightly, whereas *PDS* expression increases more than 3-fold (not shown, ref. 8).

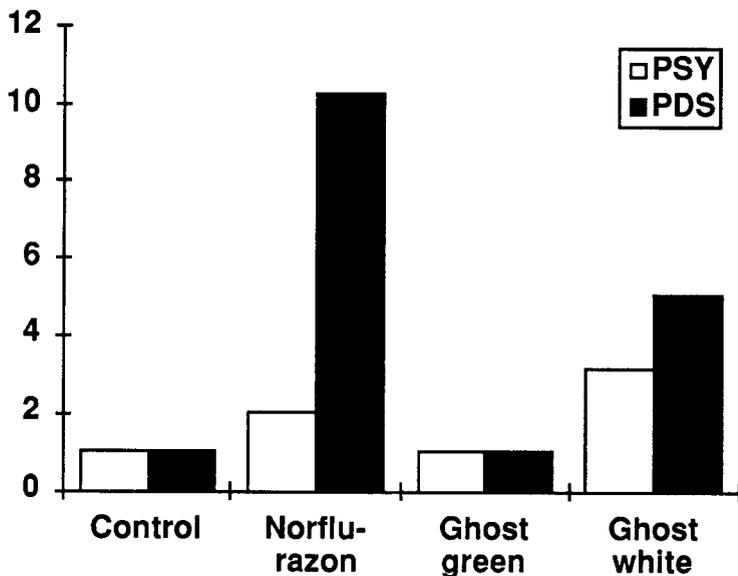


Fig. 3. *PSY* and *PDS* expression in seedlings.

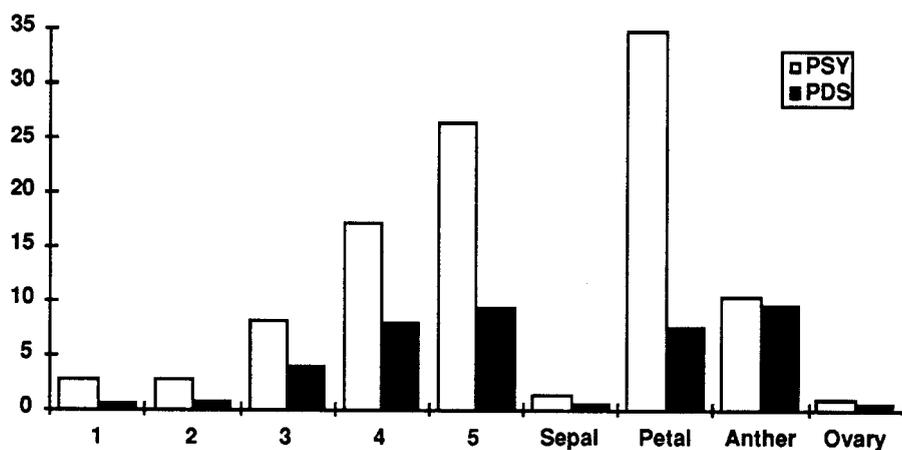


Fig. 4. *PSY* and *PDS* expression in flower.

In early stages of flower development *PSY* expression is slightly higher than in mature leaves, whereas *PDS* is expressed at basal levels. Expression of both genes is induced approximately 10-fold during flower development (Fig. 4). Dissection of flowers into the four organ whorls indicates that this induction is due to high expression levels in chromoplast-containing (petals and anthers) organs, while basal levels of expression are maintained in sepals and ovaries.

We measured expression of *PDS* and *PSY* during fruit ripening, when the color of the pericarp changes from green to red as chlorophyll is degraded and carotenoids accumulate. During this process chloroplasts differentiate into chromoplasts, chlorophyll content declines to undetectable levels, and carotenoid content increases. From immature green to orange, *PSY* is induced >25-fold but *PDS* mRNA levels increase less than 3-fold (Fig. 5).

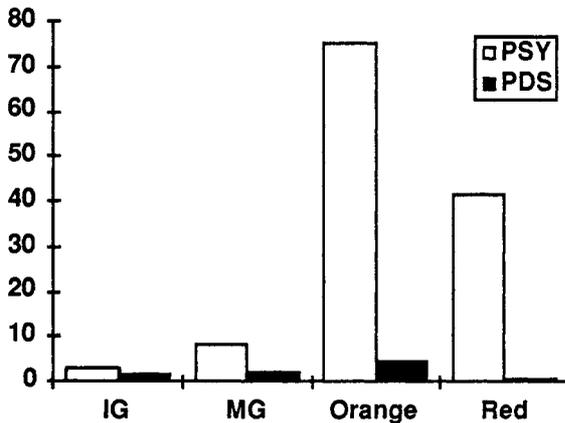


Fig. 5. *PSY* and *PDS* expression in Fruit. Green stages: immature (IG) and mature (MG).



Fig. 6. Genome mapping of *PDS* and *ghost*.

PSY and *PDS* are maximally expressed in flowers and fruits and at very low levels in roots. Transcript levels in petals are higher than in sepals. Levels of mRNA in anthers are also higher than in sepals for both *PDS* (>17-fold) and *PSY* (>7-fold). The difference in mRNA levels between petals and roots is >316-fold for *PSY* and >94-fold for *PDS*. Expression of both genes in stems is slightly lower than in mature leaves.

MAPPING

To determine whether the *ghost* mutant of tomato corresponds to a lesion in *PDS*, we mapped this gene in the tomato genome. An *Xba*I polymorphism present in this gene between *Lycopersicon esculentum* and *L. peruvianum* was scored in a population of 67 F₂ individuals from a *L. esculentum* × *L. peruvianum* cross (K. Alpert, personal communication). Segregation of the corresponding alleles showed no significant deviation from the expected 1:2:1 ratio ($\chi^2=2.16$). According to the linkage data, *PDS* maps to chromosome 3, at approximately 10.2 and 14.5 centimorgans from markers TG94 and TG152, respectively (Fig. 6). Therefore, this gene does not correspond to the *GHOST* locus, which maps to chromosome 11.

CONCLUSIONS

By using RT-PCR we were able to demonstrate regulation of *PSY* and *PDS* by flower and fruit development and by photooxidation. We have also shown that expression of both genes is not affected by de-etiolation, a process that results in a 4-fold increase in carotenoid levels. Analysis of *PSY* and *PDS* promoters is in progress to elucidate the molecular basis of these control mechanisms.

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