Improving provitamin A (carotenoid) content of rice endosperm

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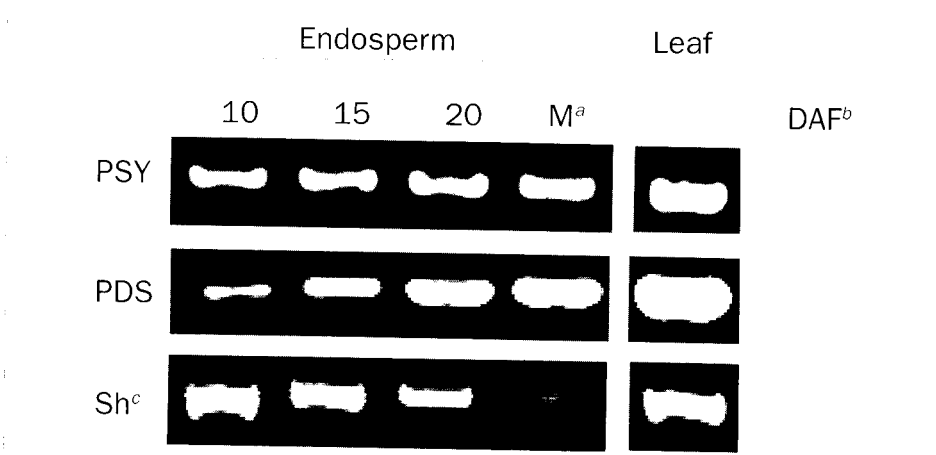
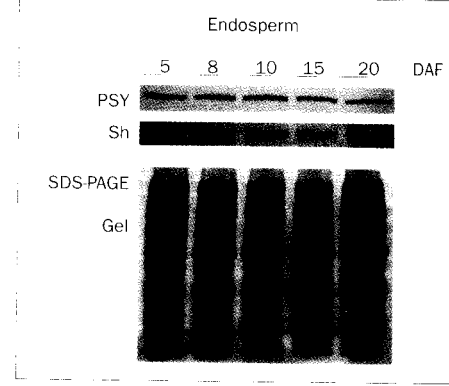
Genetic engineering is a feasible approach to alter or improve carotenoid content of endosperm, an agronomically valuable tissue. However, to plan a strategy for engineering rice endosperm, we must identify the biosynthetic block preventing carotenoid accumulation. Three alternative possibilities are being considered: 1) genes encoding one or more of the biosynthetic enzymes may not be expressed in the endosperm, 2) these enzymes are expressed but are not functional, and/or 3) a limitation



1. RT-PCR analysis: PSY and PDS transcribed throughout rice endosperm development. [a]M = mature endosperm. [b]DAF = days after flowering. [c]Sh = Sucrose synthetase.



2. Western blot analysis: PSY protein expressed throughout rice endosperm development.

exists in precursors available to the pathway. Rice endosperm lacks both end products of the carotenoid biosynthetic pathway and any intermediates, suggesting that biosynthetic enzymes may not be expressed. Only geranylgeranyl diphosphate (GGPP), a substrate of the first enzyme specific to the carotenoid biosynthetic pathway, is present. GGPP is a precursor common to other pathways, such as the gibberellic acid biosynthetic pathway.

We developed gene and protein probes to test the expression of the PSY and PDS, the first two enzymes specific to the pathway. Genes encoding both enzymes were each mapped to a single genetic locus in rice and in maize and behaved as single copy genes by Southern blot analysis. Reverse transcriptase-polymerase chain reaction showed transcripts in the leaves and endosperm. In developing rice endosperm, the PSY transcript was constant, whereas the PDS transcript increased over time (Fig. 1). Using a polyclonal antiserum raised against maize PSY, Western blot analysis showed PSY to be constantly expressed in developing rice endosperm (Fig. 2). We used a starch gene encoding sucrose synthetase to compare the transcript and protein analyses. Finally, high-pressure liquid chromatography analysis of a rice carotenoid mutant showed phytoene (a carotenoid intermediate) in the leaves and embryos but not in the endosperm.

Several factors likely cause the carotenoid deficiency in rice. Genes encoding PSY and PDS, the first two biosynthetic enzymes, are expressed in developing rice endosperm. However, the absence of intermediates, even in rice mutants blocked in the pathway, suggests that the endosperm PSY does not function or that precursors feeding the pathway are limiting. Also, the level of PDS transcripts was not constant, suggesting the possibility of discordant expression of enzymes at inappropriate levels or times during endosperm development, which would contribute to the general problem of carotenoid deficiency. ■