



## DIFFERENTIAL EXPRESSION OF CAROTENOGENIC-RELATED GENES IN TWO ANNATTO VARIANTS

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*Carotenoids, DOXPS, PSY.*

**Introduction.** *Bixa orellana* L. is a neotropical native plant with high carotenoid pigment content in its seeds. It is the only known source of bixin, a widely used pigment in the food industry (1). Carotenoids in plants are produced by the isoprenoid pathway where the DOXPS enzyme is the first specific step in the route and the PSY enzyme is the first step in the carotenogenic portion of this pathway (2). Expression behavior of these enzymes was evaluated during flower and fruit development stages in two annatto variants with dissimilar phenotypic characteristics (white flower-green pods and pink flower-red pods).

**Methodology.** Tissues from adults plants (leaf, flower buds, open flowers, immature and mature fruits, and immature and mature seeds) of two annatto (white flowers and pink flowers) variants were used (Fig. 1). DOXPS and PSY homologues probes were isolated from DNA genomic using degenerate primers designed to align conserved regions of plant genes. The Ribonuclease Protection Assay (RPA) methodology was chosen for expression analysis using the isolated probes because of the high concentration of metabolites present in annatto. A ribosomal antisense riboprobe of 158 bp corresponding to the 28S subunit from *Camptotheca acuminata* cDNA (U42789) was used as a control to determine the amount of RNA utilized in each reaction. A fosforimager device (Bio Rad Hercules, CA) was used to quantify each transcript's signal amount. Pigment analysis was done according (4).



Fig. 1. Annatto varieties (Left White flower, Right Pink flower).

**Results and discussion.** Two cDNA fragments from annatto, one of 582-bp and the other of 607-bp, were cloned and sequenced. Both showed high homology to DOXPS (AY724809) and PSY (AY613925) from other plant protein sequences. The expression of messenger encoding DOXPS enzyme reached its highest level during early fruit development, before the most significant carotenoid accumulation in both variants. However, this expression was stronger in the pink flower variant tissues. Messengers encoding to the PSY enzyme were accumulated in different

tissue types in proportion to the carotenoid accumulation observed in later development stages (Fig. 2). Pigment analysis results showed constant accumulation of total carotenoids and bixin in both variants, independent of analyzed tissue class. Major carotenoid accumulation was found in mature seeds from the pink flower-red nod variant.

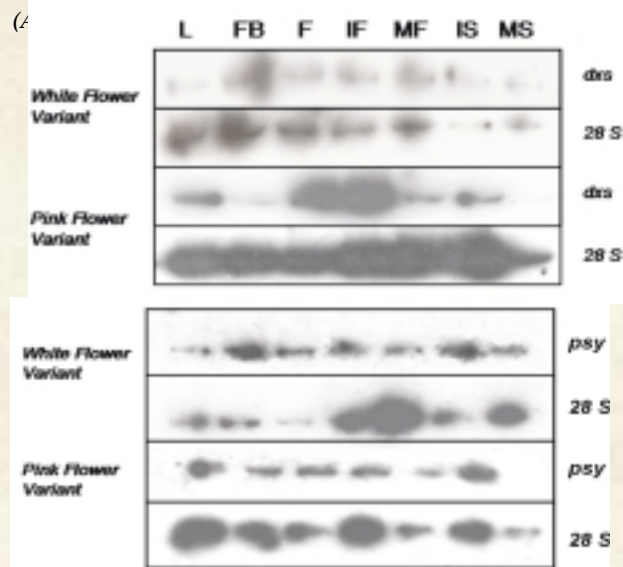


Fig. 2. Ribonuclease protection assays. RNAase-protected fragments were resolved in 5% nondenaturing polyacrylamide gels. (A) DOXPS probe (B) PSY probe L: leaves, FB: flower buds, F: flower, IF: immature fruit, MF: mature fruit, IS: immature seeds, MS: mature seeds.

**Conclusions.** The results of this study suggested a coordinated action between the DOXPS and PSY enzymes involved in differential carotenoid accumulation in the two annatto variants.

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