Complex but Coordinated Biochemistry Underlies Floral Nectary Function in Ornamental Tobacco

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Abstract
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Disciplines
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Comments
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Complex but Coordinated Biochemistry Underlies Floral Nectary Function in Ornamental Tobacco

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The ornamental tobacco floral nectary gland is a remarkable organ that functions both to attract visiting pollinators and to protect the gynoecium from microorganisms vectored to the flower by these pollinators. We have documented an integrated biochemistry that underlies these biological functions. Ornamental tobacco floral nectaries undergo dramatic changes in both size and color as the flowers mature. The change in color from lime green at early stages to bright orange at maturity is due to the accumulation of the potent antioxidant β-carotene (Figure 1). The change in size is accompanied by a major accumulation of starch granules in nectary amyloplasts. We isolated and characterized starch from nectaries throughout development. At early stages, little starch is present in the nectary. With development, both starch quantity and complexity increase until a maximum amount of starch is present at Stage 9, when approximately 20% of the total nectary mass consists of starch. After this point, nectary starch declines dramatically, suggesting its conversion to free sugars.

RT-PCR analysis of 18 starch metabolic genes revealed that starch anabolic genes, but not starch catabolic genes, are expressed in early nectary development; then a major metabolic shift occurs just before Stage 9 that down-regulates the anabolic genes while the starch catabolic genes are up-regulated. Quantifying levels of sugar in nectar, we find that the total amount of carbohydrate stored in nectary starch at Stage 9 is roughly equivalent to the carbohydrate present in nectar at anthesis; however, after anthesis, four- to five-fold more sugar is secreted into nectar, suggesting that a second mechanism is responsible for this larger nectar flow. Investigating the role of photosynthate transport using radio-labeled sugars revealed that the nectary is the strongest sink of all floral organs and that photosynthate transport into nectar does not occur prior to anthesis but is very strong in the hours following anthesis. We propose that two processes, starch degradation and rapid photosynthate transport, are the primary determinants of sugar composition in floral nectar. We also evaluated accumulation of β-carotene and ascorbic acid that provide important antioxidant activity both in the nectary and in soluble nectar as the highly oxidative Nectar Redox Cycle is expressed. Patterns
of accumulation of these compounds reveal that they are synthesized in nectaries at early developmental stages, but they do not accumulate to high levels until after the metabolic shift, suggesting a link between starch breakdown and antioxidant production. RT-PCR analysis of 13 \( \beta \)-carotene and ascorbic acid biosynthetic genes confirmed expression at early developmental stages and established that antioxidant accumulation is not directly regulated by transcription. By feeding radiolabeled sucrose to Stage 9 flowers, we demonstrated isotope incorporation from sugars into \( \beta \)-carotene. We propose that the biosynthesis of these antioxidants is governed by the availability of substrate molecules arising from starch degradation following the Stage 9 metabolic shift (Figure 2). We are currently investigating the methylerythritol phosphate (MEP) pathway as a possible link between these sugar substrates and antioxidant production.

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**Figure 1.** Development of the ornamental tobacco floral nectary (at base of gynoecium) showing increase in size and change in coloration.

**Nectary Metabolic Shift**

**Figure 2.** During development (Stage 6), the nectary stores sugars as starch. At anthesis (Stage 12), starch is broken down to provide sugars for nectar and substrates for antioxidants important in the nectar redox cycle.