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RESEARCH BRIEFS:

A hairy tissue produces vanillin

ABSTRACT

A unique secretory tissue produces the most important flavoring agent. Vanillin accumulates in the secretion around the seeds in the mature fruit of the vanilla orchid. A unique secretory tissue that is composed of closely packed unicellular hairs is located in the three gaps between the placentas along the central fruit cavity. These cells contain enzymes that are involved in vanillin biosyntheis and seem to be responsible for vanillin secretion.

INTRODUCTION

Vanillin, the most important flavor compound in the food and flavor industry and one of the oldest flavoring agents, is obtained from the bean-like fruit of the orchid *Vanilla planifolia* Andrews. The high demand for natural rather than synthetic vanillin and the difficulties in meeting this demand by its limited production have led to a dramatic increase in the price of vanilla beans. The manipulation of vanillin biosynthesis may be a way to increase the production of the compound. However, its site of synthesis in the plant is poorly understood. Here we show that a unique internal fruit tissue is responsible for the production and secretion of vanillin.

Vanillin, which is stored in the fruit as glucovanillin, starts appearing in the fruit 3–4 months after pollination and is synthesized in the developing fruit for the next 4–5 months. The vanilla fruit is harvested green and flavorless and is then cured for 6–9 months, under conditions of high heat and humidity, during which period the vanillin is enzymatically released from glucovanillin. Whereas upon harvest glucovanillin is 10–15% of the green bean dry weight, the commercial cured "beans" contain only 1–3% vanillin, apparently because of losses during curing. The discovery of the site of vanillin biosynthesis may help in understanding and improving the curing process.

VANILLIN BIOSYNTHESIS

It is believed that vanillin (4-hydroxy-3-methoxybenzaldehyde) (Fig. 1) is a product of the phenylpropanoid pathway of plant secondary metabolism (Havkin-Frenkel et al., 1999; Dignum et al., 2001).

A key reaction of the branch pathway leading to vanillin and many other related benzoic acid derivatives is the shortening of the three-carbon side chain of a hydroxycinnamic acid derivative to a single carbon moiety. However, other pathways for vanillin production have also been proposed (Zenk 1965; Funk and Brodelius, 1990a, b). Recently we provided evidence

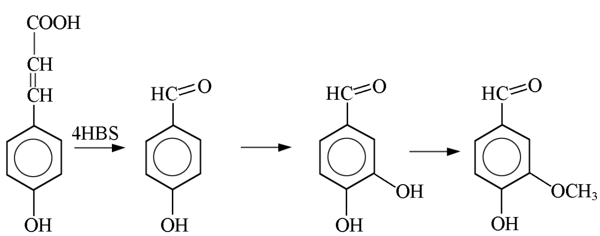

Fig. 1. Proposed vanillin biosynthetic pathway, from 4-coumaric acid (1) via 4-hydroxybenzaldehyde (2) and 3,4-dehydroxybenzaldehyde (3) to vanillin (4).

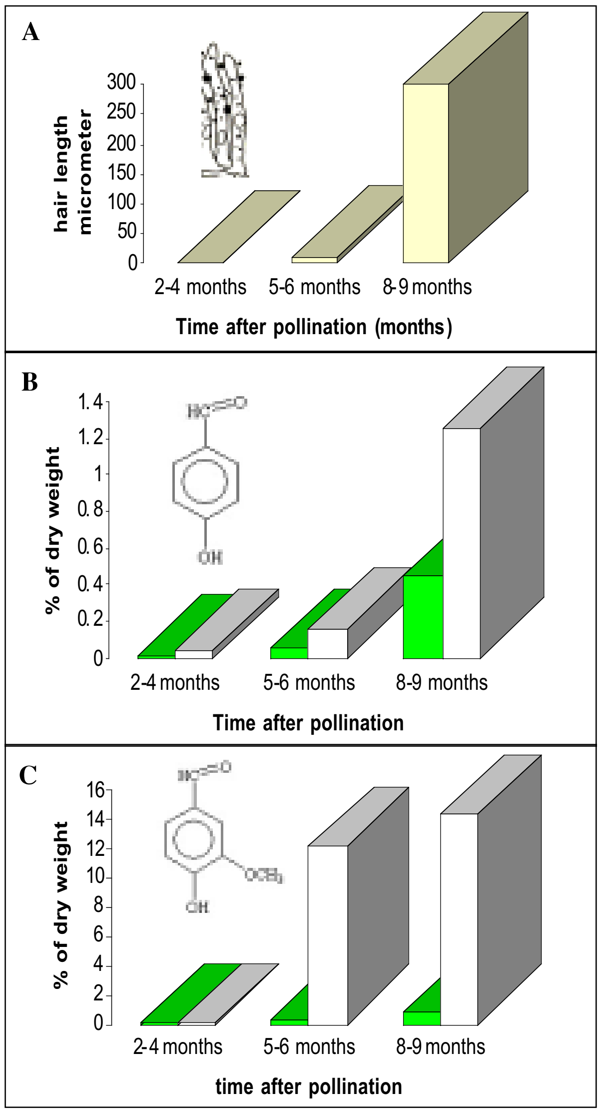

Fig. 2. Fruit characteristics at various stages after pollination. A. The length of glandular hair cells. B. 4-hydroxybenzaldehyde content in the inner (white) and outer (green) parts of the fruit. C. Vanillin content in the inner (white) and outer (green) parts of the fruit.

that vanillin is produced from 4-coumaric acid via 4-hydroxybenzaldehyde and 3,4-dihydroxybenzaldehyde (Havkin-Frenkel et al., 1996, 1999). We showed that the first step from 4-coumaric acid to 4-hydroxybenzaldehyde is mediated by a putative 4-hydroxybenzaldehyde synthase (4HBS) (Podstolski et al., 2002). These findings were also supported by feeding experiments entailing the application of ^{14}C labeled tyrosine and phenylalanine to vanilla embryo culture and vanilla bean on the vine. Label was incorporated into glucovanillin as well as into intermediates of the vanillin biosynthetic pathway. The results of feeding with unlabeled intermediates further confirmed the proposed pathway (Havkin-Frenkel et al., 1996; Herz, 2000).

LOCALIZATION OF VANILLIN

Swamy (1947) described the structure of the vanilla bean and proposed that vanillin is secreted "in tissues around the seeds". Jones and Vicente (1949) also pointed out that most of the vanillin and other compounds involved in vanilla flavors were found in the middle part of the bean around the seed, but until now the exact site of vanillin synthesis has been unknown.

In a comprehensive study of vanilla fruit development, we found that vanillin is specifically present in the non-photosynthetic parenchyma cells of the endocarp. Separation of these "white" inner fruit portions from the outer "green" exocarp revealed that the former contains 95% of the total vanillin in the vanilla pod (Fig. 2C) and that this parenchymatic tissue also contains proposed intermediates of vanillin biosynthesis, including 4-coumaric acid, 4-hydroxybenzaldehyde (Fig. 2B), and 3,4-dihydroxybenzaldehyde. The extracted glucosides were hydrolyzed to the corresponding aglycones with almond β-glucosidase and identified with HPLC coupled to LC-MS, as previously described (Podstolski et al., 2002).

We also used catechin-HCl, which binds to various phenolic compounds including vanillin, to locate the accumulation of vanillin in the developing fruit more accurately. Both

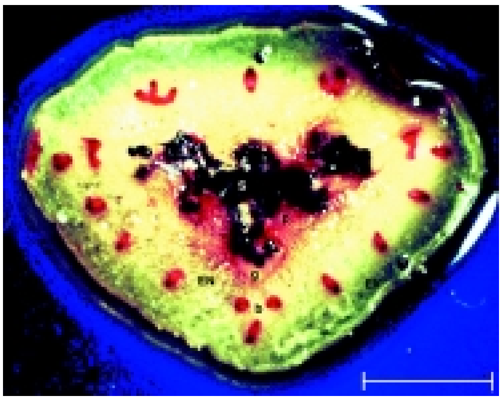

Fig. 3. Cross section of a mature green vanilla fruit after catechin-HCl staining. Vanillin stained red in the placenta (p) and neighboring endocarp (EN) cells, and also in the secreted matrix that surrounds the seeds (s) in the fruit cavity. The fruit vascular bundles that are rich in lignin also stained red (b). EX = exocarp. g = secretory tissue. Scale bar = 5 mm.

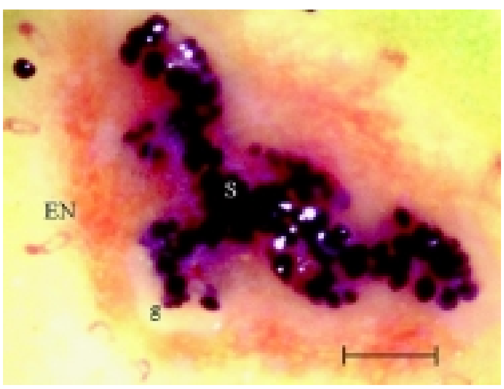

Fig. 4. The central portion of the fruit, showing a dense catechin-HCl staining of the secreted matrix in the fruit cavity around the seeds (s), and a weaker staining in endocarp cells (EN) adjacent to the cavity. g = secretory tissue. Scale bar = 2 mm.

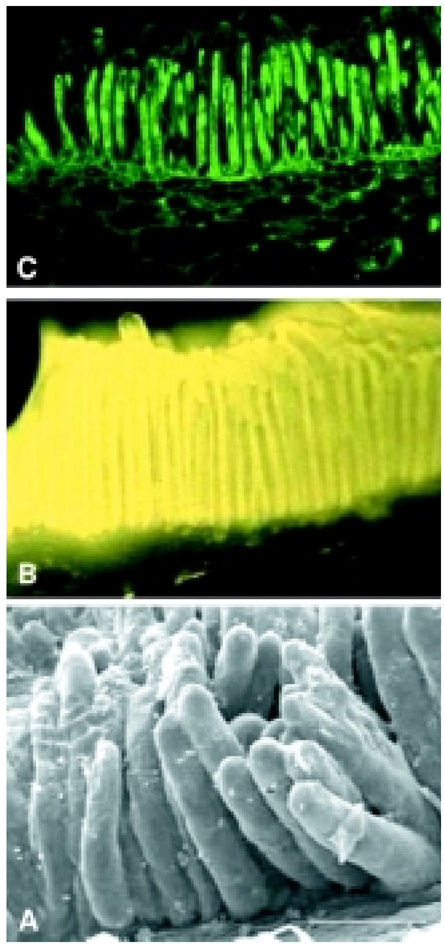

Fig. 5. The vanillin-producing hairs. C. Confocal micrograph with immuno-localization of the putative 4-hydroxybenzaldehyde synthase that is located in the cytoplasm of the hairs. B. Epifluorescence of the hairs under UV excitation. A. Scanning electron micrograph (scale bar = 150 µm).

the placenta and the adjacent endocarp parenchymatic cells stained red (Fig. 3), indicating the presence of vanillin and intermediates of its biosynthesis in these tissues.

Catechin-HCl also stained the densely packed secreted matrix that accumulates in the fruit cavity (Fig. 4). We could clearly see a staining gradient in the endocarp from the fruit cavity outwards. As determined by this method, vanillin started to accumulate after 3 to 4 months of fruit development.

However, no staining could be seen in the longitudinal strips of brilliant whitish secretory tissue located in the gaps between the placentas along the central fruit cavity (Figs. 3, 4).

VANILLIN-PRODUCING CELLS

The longitudinal strips of secretory tissue are composed of closely packed unicellular hairs that develop by elongation of the inner epidermal cells facing the fruit cavity (Fig. 2A,B). Each mature hair is ca. 300 µm long (Fig. 5).

The unicellular hairs contain a dense cytoplasm with a granular appearance (Fig. 6) and are closely packed together forming the white "hairy tissue" that fluoresces under UV excitation (Fig. 5B) due to phenolic substances that it secretes to the apoplast. The hairs secrete copious amounts of the sticky substance that accumulates in the fruit cavity around the seeds (Figs. 4, 6).

Immuno-localization of a cysteine-protease-like protein that appears to act as a 4-hydroxybenzaldehyde synthase, the first enzyme in the vanillin biosynthetic pathway (unpublished results), revealed a location mostly in the cytoplasm of the secretory hairs (Fig. 5C). Thus, it seems that glucovanillin biosynthesis takes place in the hairs.

These findings, which combine structural, physiological, biochemical, and immunological data, are the first to reveal the presence of special structures that are involved in the production and secretion of vanillin in the fruit of the vanilla orchid. The discovery of the site of vanillin production may provide scientists an extra tool to understand the curing process and thereby to improve it.

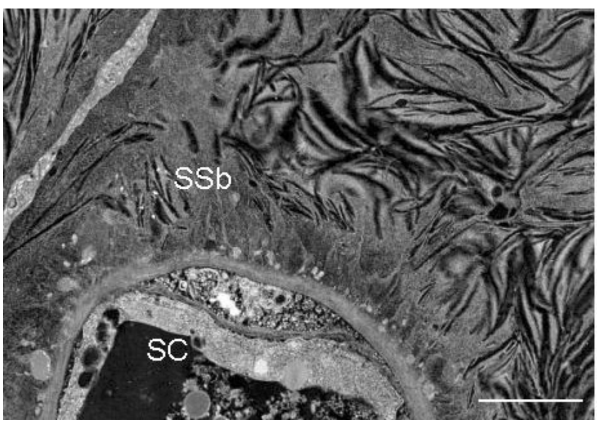

Fig. 6. Transmission electron micrograph showing a portion of a secretory hair cell (SC) with dense granular cytoplasm, and the secreted substance (SSb) in the fruit cavity. Scale bar = 2.5 µm.

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