Vanilla Enters the World of Genomics

A progress report

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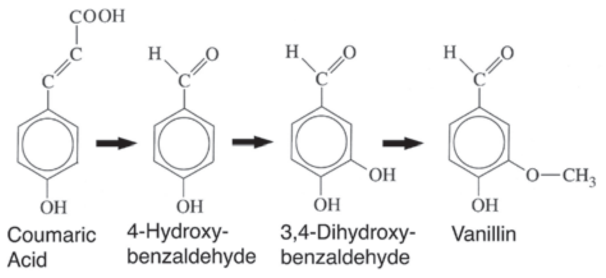
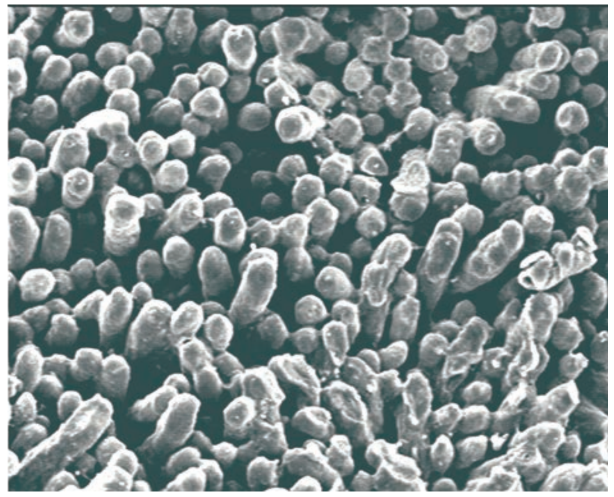
The shape, size and properties of any organism—animal, plant or microbe—are determined by the information encoded in its genome (DNA) and how the readout of this information is modulated by its interactions with the environment. The last decade has provided numerous examples of how genomic information has guided and informed our understanding of human, animal and plant health and disease. However, because of the costs involved, application of genomic approaches was previously limited to humans and the major agricultural plants and animals. Within the last few years, the development of new DNA sequencing technologies has revolutionized the study of crop plants. New technologies are now available that provide high-throughput DNA sequence coverage at low cost, making their application to smaller crops possible. Two research groups are currently applying these new technologies to *Vanilla planifolia* to study some of the important issues with *Vanilla*, such as disease susceptibility and vanillin biosynthesis.

At Rutgers, Daphna Havkin-Frenkel and Faith Belanger are currently pursuing characterization of the vanillin biosynthetic pathway. Although vanillin is one of the most important flavor compounds in the world, its biosynthetic pathway is still not known. Understanding the vanillin biosynthetic pathway will be important in future efforts to improve vanilla flavor. Based on the vanillin structure, several possible pathways have been proposed, but none have been confirmed experimentally.[1] One of the proposed pathways is shown in **F-1**. Confirmation of any biosynthetic pathway requires purification and characterization of the enzymes involved. One of the reasons that the biosynthetic pathway for a molecule as simple as vanillin is not yet known is the difficulty and expense in obtaining appropriate biological material for research. Vanillin is synthesized in abundance only in some species of *Vanilla*, and only in specific tissues at a specific developmental stage of the developing pods. The specialized hair cells near the center of developing *Vanilla* pods have been proposed as the site of vanillin biosynthesis (**F-2**).[2] The limited tissue availability of these specialized cells makes standard biochemical approaches to characterization of the vanillin biosynthetic pathway impossible.

A proposed biosynthetic pathway for vanillin **F-1**



Scanning electron micrograph of the hair cells of a developing *Vanilla* pod **F-2**



As an alternative to direct enzyme purification from the tissue of interest, DNA sequencing of expressed genes in the target tissue followed by characterization of likely genes has become a successful approach. In this approach, RNA (which represents the genes being expressed in that tissue) is isolated from the tissue of interest and converted to DNA, which is then sequenced. Pathway genes identified through DNA sequencing can be then be cloned into bacteria and the bacteria will produce the enzyme encoded by the cloned gene. The pathway enzymes can then be purified from the bacteria instead of from the original tissue, in this case the *Vanilla* hair cells.

The DNA sequencing approach can be used to provide clues as to what genes are important in a particular pathway by analyzing the abundance and tissue location of a particular sequence. For example, since vanillin is synthesized only in specialized cells in the developing pod, genes that are expressed exclusively in those specialized cells are likely to be involved in the biosynthetic pathway. Using standard sequencing technology, researchers obtained some sequence data from genes expressed in the pod hair cells, the likely location of vanillin biosynthesis, and characterized two candidate *O*-methyltransferase genes. One of these was highly expressed in the hair cell tissue and is likely to be involved in the vanillin biosynthetic pathway.[3]

The relatively low cost of next-generation sequencing technologies makes it feasible to obtain massive sequencing datasets of expressed genes from different tissues and developmental stages of *Vanilla* pods. Comparisons of abundance of expressed genes at the different developmental stages can be used to identify the genes involved in the biosynthetic pathway. Havkin-Frenkel and Belanger are collaborating with Richard Dixon from the Noble Foundation to obtain sequence data from different pod tissues harvested at different times of pod development. Those genes whose expression is correlated with the timing of synthesis of vanillin and that are mainly expressed in the hair cells will identify the pathway genes, which will be further characterized by enzyme purification from bacteria. The researchers expect that this massive sequencing approach will allow them to finalize the vanillin biosynthetic pathway.

Sharman O'Neill and Christopher Town are using new sequencing technologies to better understand fruit development and pod shattering and to identify genes that are important for disease resistance. Through the Vanilla Sustainability Project, funded by General Mills, they are embarking on a first exploration of the *Vanilla* genome. The *Vanilla* genome is quite large, comparable in size to the human genome. To reduce the scope of the project, their first effort is directed at generating sequence of just the *Vanilla* genes, which represent just a small fraction of the entire *Vanilla* DNA complement. From this information, it will be possible to make a catalog of the entire *Vanilla* gene repertoire including those involved in fruit development and flavor generation as well as those that might condition disease resistance. During the course of this work, they will also explore the genetic repertoire of *Vanilla bahiana,* which shows greater *Fusarium* resistance and might provide impetus for introgression of resistance traits into *V. planifolia*.

Vanilla has been vegetatively propagated for the past 300 years; thus many plantings of this crop are very closely related. A critical aspect of the future of *Vanilla* is to introduce new germplasm and traits into the crop. In addition to developing the gene catalogs described above and monitoring how their expression changes during fruit (pod) formation, O'Neill and Town will also survey the diversity of extant *Vanilla* cultivars. Using a technology known as genotyping-by-sequencing that can cost-effectively sequence just a fraction of each genome, they will survey cultivars from around the world to determine their relatedness. By combining this information with their varietal characteristics, it should be possible to home in on those varieties that are the best candidates for crop improvement, be it flavor, disease resistance or stress tolerance.

The next few years will be very exciting for *Vanilla* research. DNA sequencing often results in unexpected insights into an organism's physiology and evolution and the authors expect the same for *Vanilla*.

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References

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