NMR-based metabolomic characterization of Vanilla planifolia

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Summary

Vanilla planifolia belongs to the Orchidaceae family and is the major source of natural vanilla flavour which is used in dairy products, beverages, bakeries and perfumes. From eight to nine months after manual flower fecundation, the vine bears mature pods which will follow a curing process in order to release the pleasant flavour. Madagascar is the largest producer of vanilla and leaves little economic potential for other small producers of vanilla. Vanilla pods from a higher quality could help these small producers to be more competitive on the global market.

The aim of the research presented in this thesis is to increase the production of flavour compounds in the pods of the orchid *V. planifolia*. To achieve this goal, a metabolomic analysis of *V. planifolia* has been performed in order to understand the mechanisms implied in the physiology and phytochemistry of the plant. The metabolomic analysis has been performed principally on the green pods and leaves of *Vanilla* by ^{1}H NMR spectroscopy.

First objective has been to follow the evolution of primary and secondary metabolites in developing *V. planifolia* pods (**Chapter 3**). ^{1}H NMR spectroscopy allowed the detection of organic acids, sugars and phenolic compounds. Furthermore, this technique permitted the detection and identification of both aglycones and glucosides of the phenolics present in vanilla pods. Vanillin (the major phenolic compound of vanilla) and its glucoside the glucovanillin have already been clearly identified in the developing pods. It has been shown that vanillin, glucovanillin and sucrose increase during the development of the pods, whereas the glucose and organic acids content decreases. The metabolomic analysis also showed that bis[4-(β-D-glucopyranosyloxy)-benzyl]-2-isopropyltartrate (glucoside A) and bis[4-(β-D-glucopyranosyloxy)-benzyl]-2-(2-butyl)-tartrate (glucoside B) could be precursors of glucovanillin and vanillin.

Metabolomic analysis of the mature pods of *V. planifolia* from different accessions showed that this method can be used for the selection of elite accessions based on the metabolic profile (**Chapter 4**). Furthermore, it has been observed that the pods length is positively correlated to the glucovanillin content. Thus, selection of accessions producing longer vanilla pods should permit an increase in vanillin content of the cured vanilla pods.

Metabomics has also been applied to the analysis of vanilla leaves (**Chapter 5**). This method has enabled insight into the effect of leaf age and collection time on the metabolic profile of the leaves. In addition, it has been observed that the two glucosides (A and B) which were present in the green pods are also present in the leaves. It could then be interesting in the future to see if these compounds are transported from the leaves, and if a higher content in the leaves could induce and increase the levels of phenolic compounds in vanilla pods. The Cymbidium mosaic virus is a virus easily transmitted in vanilla.

In **Chapter 6**, metabolomic analysis of the virus effect on metabolic profile of vanilla leaves has shown that the virus infection is correlated to an increase of sugar content and a decrease of the phenolic content. Nevertheless, in order to obtain a complete understanding of the mechanism implied with the virus infection, further studies will have to focus on the early metabolic changes induced by the virus infection. Furthermore, it has been observed from the field that *V. pompona* is more resistant to CymMV, and presents less necrotic symptoms compared to *V. planifolia* and other related species. Metabolomic analysis has shown that *V. pompona* leaves possess relatively a higher phenolic content then leaves from other species. These compounds could be responsible of the resistance of the *V. pompona* accession.

Additionally, in **Chapter 7**, shoot formation during *V. planifolia* callus differentiation has been investigated by ^{1}H NMR spectroscopy. Analysis of the spectra obtained suggest that there is an early stimulation of several metabolic pathways including mobilization of sucrose, glycolysis and phenolic compound synthesis, and amino acids synthesis among others, to assemble the photosynthetic machinery in the cells. Metabolomic analysis showed that at a very early stage of plant development, coumaric acid glucosides A and B are already produced. Investigation into the involvement of these compounds in the biosynthesis of vanillin could be conducted in the future on vanilla callus samples, which may lead to interesting results.