NMR-based metabolomic characterization of Vanilla planifolia

Palama, T.L.

Chapter 8

General Discussion and Conclusions

T.L. Palama

In this project, knowledge on the metabolome of *V. planifolia* has been obtained. A metabolomic approach has been used in order to contribute to the physiological and biochemical study of *V. planifolia* plants. Indeed, the metabolome is the downstream product of gene expression. So, it reflects the functional level of the cell more appropriately than the proteome and transcriptome (Urbanczyk-Wochniak et al., 2003). It has also been suggested that in fact metabolomics may provide the most "functional" information of the "omics" technologies (Sumner et al., 2003). In order to have the most representative profile of the metabolome, detection of as many metabolites as possible in a single analysis is necessary. The ultimate goal of metabolomics is both qualitative and quantitative analysis of all metabolites in an organism or tissue. Nevertheless, simultaneous high-throughput analysis of large number of metabolites is not generally feasible. And thus, no single analytical method can accurately cover the entire metabolome. Likewise, the extensive chemical diversity of plant primary and secondary metabolites do not facilitate its comprehensive analysis. Notwithstanding, recent advances in chemometric and bioinformatic methods may enhance the global understanding of plant metabolism (Bino et al., 2004).

During the research presented in this study, nuclear magnetic resonance (NMR) has been used as a major tool to analyze the metabolome of *V. planifolia*. Major advantages of NMR spectroscopy are its non-selectivity and non-destructivity. In case of ^{1}H-NMR spectroscopy, all hydrogen-containing compounds present above the detection limit are detected. Furthermore, the structure elucidation capacities of this technique using two-dimensional (2D) and heteronuclear ^{1}H-^{13}C correlation experiments were useful to explore the metabolome of *V. planifolia*. Conclusions from the large number of data sets obtained from the many samples which have been analyzed under different conditions are then obtained after multivariate data analysis such as principal component analysis (PCA).

These metabolomic tools have been developed on *V. planifolia* to study:

- the metabolic changes occurring during the development of the green pods (Chapter 3)
- the metabolic profiles of mature green pods from different accessions of *V. planifolia* (Chapter 4)

- the effects of developmental stage, time of the day and season on the metabolic profile of vanilla leaves (Chapter 5)
- the metabolic profile of different *Vanilla sp.* leaves and the effect of CymMV infection on the metabolic profile of *in vitro V. planifolia* plants (Chapter 6)
- the induction shoot differentiation on *V. planifolia* callus (Chapter 7).

Using the non-selectivity of NMR spectroscopy, primary (sugars, amino acids and organic acids) and secondary (phenolic compounds) metabolites were both detected in *Vanilla* (**Figure 56** and **Figure 57**).

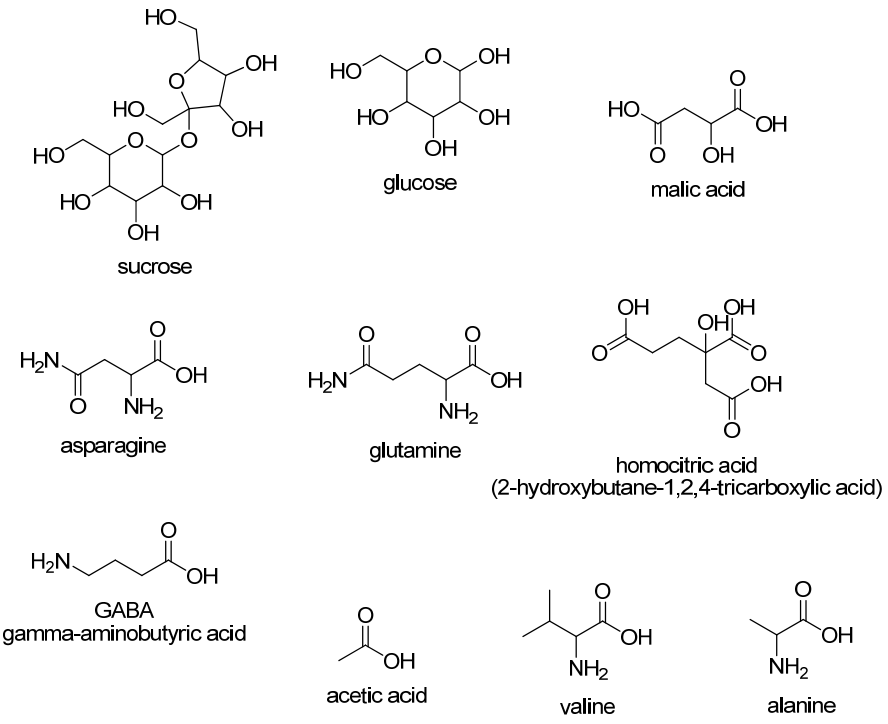

Figure 56: Primary metabolites (sugars, organic acid and amino acid) identified in *V. planifolia* by ^{1}H-NMR.

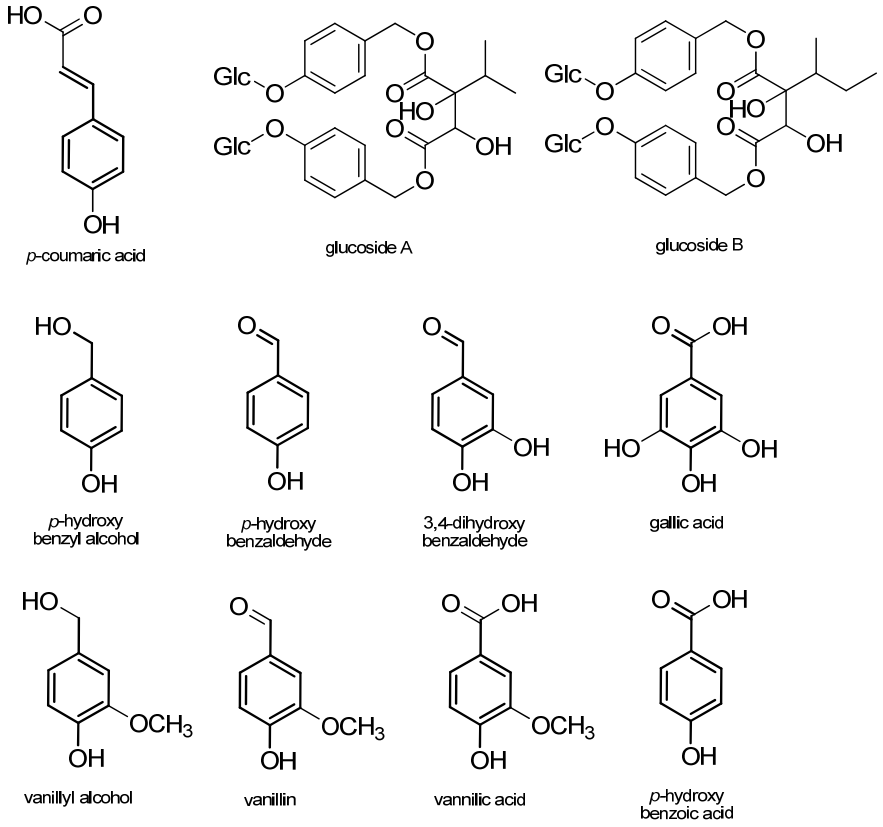

Figure 57: Secondary metabolites (phenolic compounds) directly identified in *V. planifolia* by ^{1}H-NMR and/or LC-MS. *: glucosides of *p*-hydroxybenzylalcohol, *p*-hydroxybenzaldehyde and vanillin were also detected by ^{1}H-NMR.

From the metabolomic results obtained from the different studies, it appears that the glucosides A and B could be precursors of glucovanillin and vanillin. They have been detected in large amount in developing green pods until 4-5 months after pollination. In addition, these glucosides have also been identified in the leaves of field plants, in young plants (i.e. in vitro plants) but also in differentiated tissues (organogenic calli). These results highlight their omnipresence in *Vanilla* plants. These glucosides could be locally produced in the pods and then converted to glucovanillin or they could be synthesized in the leaves and subsequently transported to the pods via the stem to follow the same conversions. Further studies have to focus on the origin of the glucovanillin precursors in the plant. These results will give significant insight in the detailed elucidation of the vanillin biosynthesis pathway.

Furthermore, it has been observed that the ratio glucovanillin over vanillin of mature green pods could vary depending on where the plants were cultivated. Analysis of a single accession of *V. planifolia* grown in two different shade houses has shown that mature green pods can contain either the glucoside of vanillin only or a mixture of both vanillin glucoside and aglycone forms. Environmental conditions during the growth of the plants might be involved in the metabolic profile. Further experiments are required to confirm these observations as it is interesting to determine which factors can affect the ratio of glucoside/aglycon of mature green pods. Moreover, at the end of the curing process, vanillin content is lower than the potential of vanillin concentration (aglycone and glucoside) present in the pod prior to the curing process. It will be interesting to determine if, in these green pods, having more vanillin under its aglycone or glucoside form could affect the composition of the cured vanilla pods.

Analysis of leaves from accessions belonging to four different species of *Vanilla* showed that the metabolomic approach combining ^{1}H-NMR spectroscopy with multivariate data analysis allows good separation between accessions from different species and even between hybrid and parents based on their metabolic profile. We have observed that CR18 (*V. pompona*) has a metabolic profile clearly different from the other related species.

We have observed that glucose and phenolic levels are higher in younger *Vanilla* leaves compared to older leaves. Phenolic compounds might have a protective role against pest and particularly in the younger leaves, whereas when the leaves are older, surface of the leaves might become stronger and become a barrier against insects. Then, phenolic compounds might be no more required in older leaves. It has been observed that glucose and phenolic compounds levels of CR18 (*V. pompona*) old leaves are higher than in other species. These phenolic compounds could explain the fact that CR18 (*V. pompona*) shows fewer symptoms upon CymMV infection.

In spite of no clear effects of CymMV observed on the leaves collected in shade house, interesting results were obtained from materials issued from *in vitro* propagation. Indeed, we have observed that virus infection is correlated to an increase of sugars content in leaf and stem samples, whereas phenolic compounds content decreases after virus infection. The absence of clear metabolic discrimination of the samples from shade house could be due to the fact that when no necrotic symptoms are observable, the CymMV do not induce major metabolic changes in the leaves. Future studies should focus on analysis of necrotic tissues and the immediate effect of viral infection.

Furthermore, we have performed a metabolomic analysis of shoot formation during *V. planifolia* callus differentiation. The results obtained show that there is an early stimulation of several metabolic pathways including mobilization of sucrose, glycolysis, phenolic compound synthesis and amino acids synthesis among others to assemble the photosynthetic machinery of the cells. Metabolomic analysis showed that at a very early stage of plant development coumaric acid and glucoside A and B are already produced. As these compounds are precursors of vanillin, callus samples could be a plant material to study the biosynthesis of vanillin precursors and further studies should be of interest.

Thus, further studies on the metabolomic of *V. planifolia* are required but it will be more interesting to integrate and monitor metabolomics with genomics, transcriptomics and proteomics data sets to obtain a global understanding of biological systems. This system biology approach will be a challenge to study and understand the biology of *V. planifolia*.