

INTRODUCTION

Sugarcane is one of the world's oldest and most important cash crops, accounting for 70% of sugar produced worldwide. Many physiological processes influence the sucrose accumulation in the stem of sugarcane and these processes are still not well known [1]. The maturation process of sugarcane involves a complex metabolic system, which begins with the photosynthetic activity in the chloroplasts of leaf cells, culminating in the accumulation of carbohydrates in stems. In order to investigate metabolic differences between the internodes in one individual, it was proposed a study of the metabolome internodes 1, 5 and 9 to search molecular factors in terms of sugar accumulation.

OBJECTIVES

Our research project aims to establish an overview of the molecular factors that control the accumulation of sucrose in sugarcane using metabolomic tools.

MATERIAL AND METHODS

Here we report the analysis of internodes 1, 5 and 9, from SP80-3280 variety (eleven month-old), submitted to normal field conditions. Plants were grown in the field between May 2012 and June 2013 and the samples were obtained from 12 individuals. Internodes metabolites were extracted from 50 mg of powder tissue, according to De Vos et al. [2] with minor modifications. Samples were analyzed by UPLC-Q-TOF-MS, in positive mode and using quercetin as internal standard. It were used six biological replicates for each tissue and three experimental replicates for each biological replicate.



Metabolite extraction
(De-Vos et al., 2007)

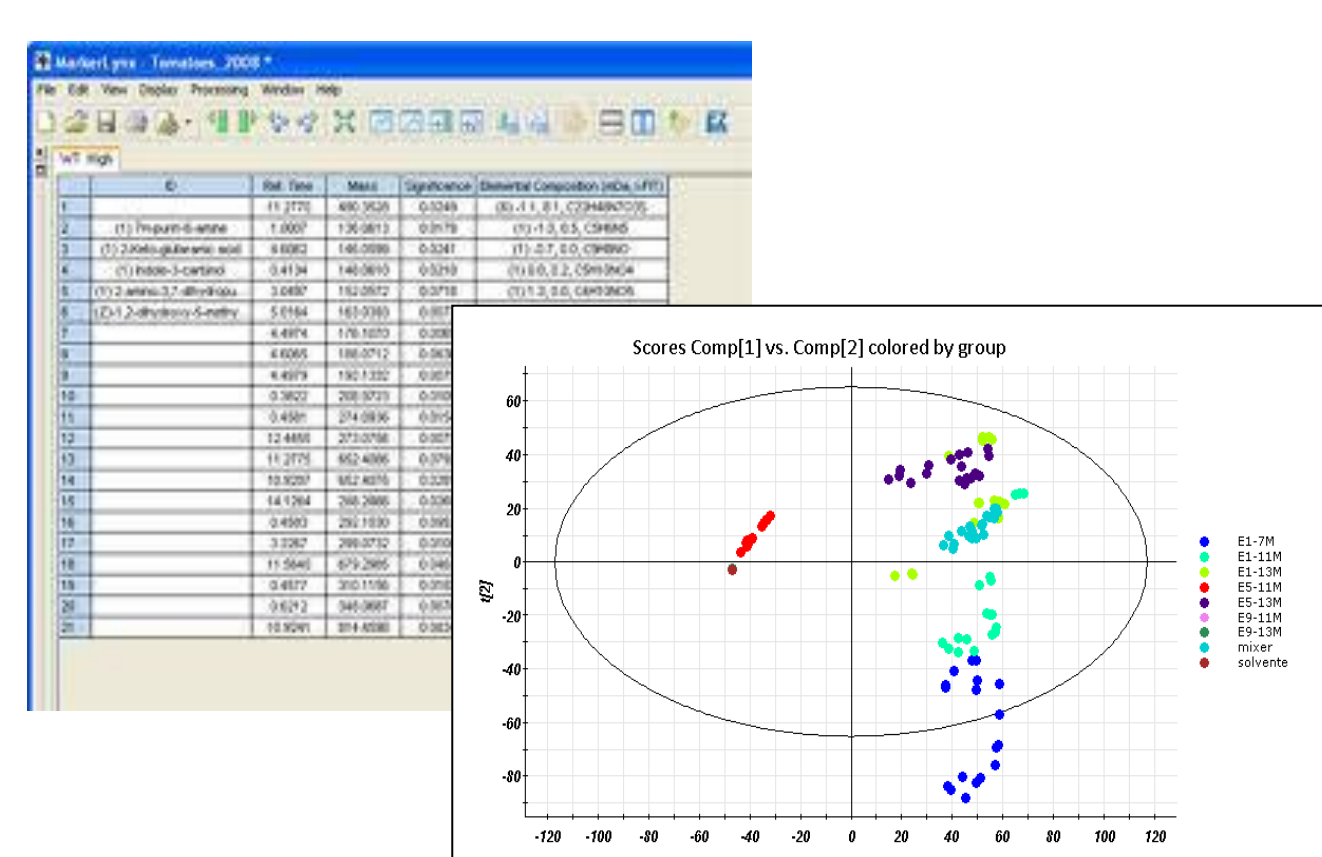


UPLC-ESI-Q-TOF-MS

Selection of internodes 1, 5 and 9 from 12 individuals (11 months-old).



Sugarcane variety SP80-3280



Data processing (MarkerLynx) and statistical analysis (EZInfo 2.0)

RESULTS

Multivariate data analysis including principal components analysis (PCA) (Figure 1) and orthogonal partial least squares analysis (OPLS-DA) were performed in MarkerLynx coupled to the plant metabolome databases. One of the comparisons was made among internodes 1, 5 and 9. PCA model demonstrated a clear separation between samples, ensured a reliable analysis.

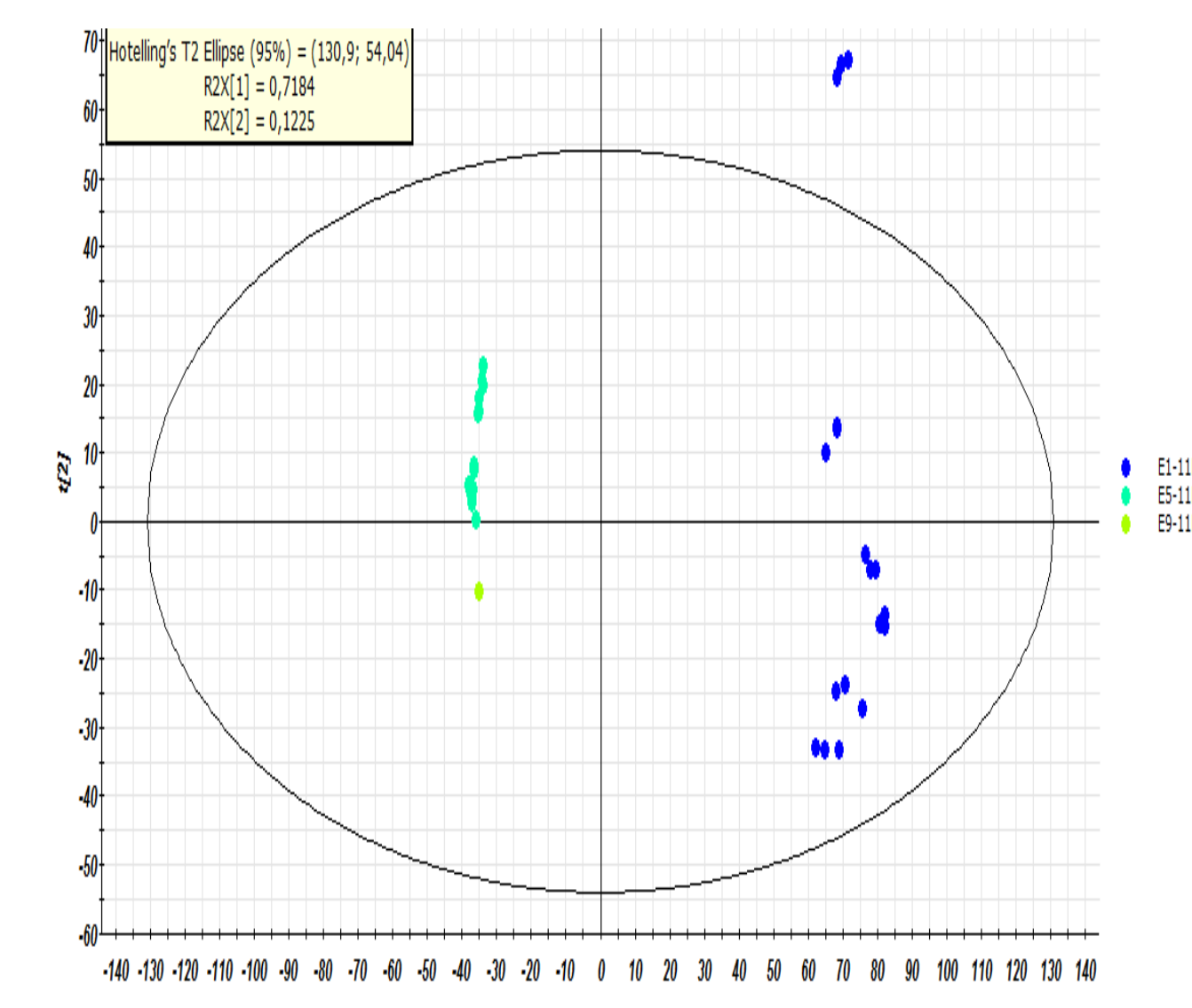


Figure 1 : PCA: metabolic difference between internodes 1 (blue), 5 (light blue) and 9 (green) in 11 months old plants.

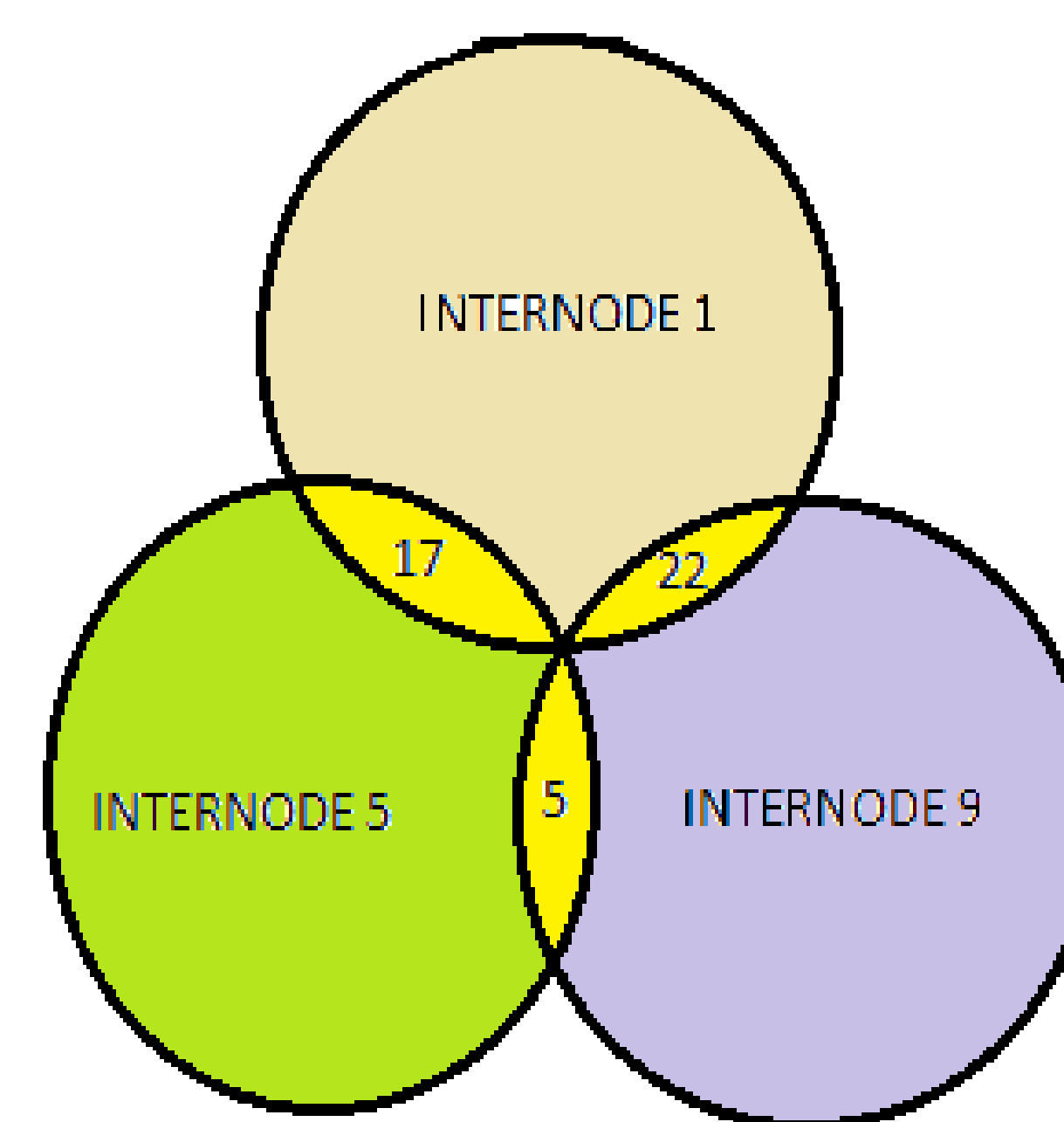


Figure 2 : Number of differential metabolites among the internodes 1, 5 and 9.

A total of 44 possible biomarkers were detected in this comparison: these metabolites were found in different concentrations or its absent/present in the internodes. 17 differential metabolites was detected in a comparison between internodes 1 and 5; 5 differential metabolites between internodes 5 and 9; and 22 differential metabolites in comparison between internodes 1 and 9 (Figure 2). The next step is to identify the differentially abundant metabolites and assemble the metabolic pathways operating in each tissue.

PERSPECTIVES

- repeating the analyzes, now in LC-MS-MS mode, to identify the differential metabolites;
- investigate the biochemical and physiological functions of these differential metabolites;
- deduce their possible function during the maturation and sugar accumulation.
- continuing comparisons, now between plant tissues at different ages.

REFERENCES

- [1] McCormick, A. J.; Watt, D. A.; Cramer, M. D. *J. Exp. Bot.* **2009**, *60*, 357-364.
[2] De Vos, R. C. H.; Moco, S.; Lommen, A.; Keurentjes, J. J. B.; Bino, R. J.; Hall, R. D. *Nature Protocols*. **2007**, *2*, 778-791.