

Challenges and Applications – Biofuels

Metabolic effect of drought stress on *Elaeis guineensis* leaves using UHPLC-MS and multivariate analysis

Jorge Candido Rodrigues Neto^{1,2}, Leticia Rios Vieira¹, Tallyta Santos Teixeira^{1,2}, Manoel Teixeira Souza Júnior^{1,3}, Patricia Verardi Abdelnur^{1,2}

¹Embrapa Agroenergy, PqEB, W3 Norte, CEP 70770-901, Brasília-DF, Brazil

²Institute of Chemistry, Federal University of Goiás, Campus Samambaia, CEP 74690-900, Goiânia-GO, Brazil.

³Graduate Program in Plant Biotechnology, Federal University of Lavras, CP 3037, 37200-000, Lavras, MG, Brazil.

jorgecrn@hotmail.com

Keywords: Biotechnology, Analytical Method, Chemometrics, Drought Stress, Metabolomics.

Introduction: Palm oil, derived from *Elaeis guineensis* is the most consumed vegetable oil in the world, with a high potential for its use in the biofuel industry [1]. The expansion of this crop in marginal areas can face challenges, such as the water deficit encountered in most parts of the globe, which can lead to an impact on the oil palm fruit harvest and palm oil production [2]. The objective of this study was the metabolic profiling characterization of palm oil leaves submitted to different drought stress.

Method: The metabolic profiling of *Elaeis guineensis* leaves was performed on 8 control samples (normally irrigated) and 8 samples without irrigation, where 4 were collected at 7 days of drought stress and the other 4 were collected at 14 days of drought stress. An extraction protocol, which uses a mixture of solvents (water, methanol and methyl tert-butyl ether), was used to extract a wide range of metabolites, from polar to non-polar, in a single shot. All samples were analyzed on a UHPLC system equipped with a reversed-phase Acquity UPLC BEH C8 column. High-resolution mass spectrometry (HRMS) was performed on a Q-TOF analyzer using an electrospray source in ESI(+)-MS and ESI(-)-MS. Acquired data were pre-processed using XCMS Online for feature detection and retention time alignment and further exported to MetaboAnalyst 4.0 for multivariate analysis. After the XCMS pre-processing, data matrices were obtained from ESI(+)-MS (3364 features) and ESI(-)-MS (2539 features).

Results: Using PCA, both ESI(+)-MS and ESI(-)-MS datasets presented a clear segregation between control, drought stress of 7 days and drought stress of 14 days, showing that the metabolism is highly affected by the lack of water. More importantly, the 7 days group was closer to the control group, when compared to the 14 days group, reflecting the metabolism changed gradually from 7 to 14 days. HCA confirmed the results, where the 14 days group was the furthest among the groups. On ESI(+)-MS heatmap, there was a higher ratio variation between up and down-regulated metabolites in each group, whereas on ESI(-)-MS the up-regulation prevailed, with metabolites having a higher concentration on the 14 days period. The mummichog algorithm [3] with putative identification was applied and, on ESI(-)-MS, 92 metabolites were identified with a fold change higher than 2, with numerous pathways affected, e.g., Pyrimidine metabolism, TCA cycle, isoleucine biosynthesis, asparagine biosynthesis, flavonoid biosynthesis, etc. On

ESI(+)-MS, 18 metabolites were identified with fold > 2, where the most affected pathways indicated were: flavonol, syringetin and leucodelphinidin biosynthesis.

Conclusion: The analysis of the metabolic profile of the present study was efficient to correlate and differentiate groups of palm oil plants submitted to different levels of drought stress. The identification of metabolites and their pathways can be used in future multi-omics analyses.

Acknowledgments: We would like to thank FINEP and CAPES for the financial support, Embrapa, UFG and IQ/UFG for the institutional support.

References:

1. GAN, P. Y.; LI, Z. D. Econometric study on Malaysia's palm oil position in the world market to 2035. *Renewable and Sustainable Energy Reviews*, 39, p. 740-747, 2014.
2. BUTT, Y. N.; FATIMA, Q.; NASAR, S.; IKRAM, J.; AKRAM, S. Drought Tolerance In Plants: A Review. *Journal of Ecology and Environmental Sciences*, 5, n. 4, p. 1-9, 2017.
3. LI, SHUZHAO et al. Predicting network activity from high throughput metabolomics. *PLoS computational biology*, v. 9, n. 7, 2013.