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Implementation of plant hormone quantification in *Arabidopsis* using UHPLC-MS/MS

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Modern plant physiology has clarified many important processes involved in plant development and crop yield. Among them, the knowledge of the mechanisms implicated in plant response against ambient fluctuations allows us to identify interesting genotypes. These processes have been reported to be regulated by molecules known as plant hormones. Cytokinins, gibberellins, auxins and abscisic acid are some examples of them. They regulate physiological processes such as apical dominance, plant transpiration by stomata closure or rooting. Although plant hormones are related to these processes, nowadays more information is needed about their signalling and action mode in plants. In this regards, our research group is constantly working in the improvement of protocols to analyse the variations of these molecules in different plants tissues grown under varied conditions. The wide experience in the study of these plant growth regulators and the use of leading technologies based on ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) have permitted us to increase the number of metabolites detected as well as simplification of purification protocol. In addition, the inclusion of micro-extraction and immuno-affinity techniques reduces the needed amount of sample per analysis. In addition, our group has an avant-garde high throughput phenotyping to continuously monitor the relative growth rate and to collect wide range of fluorescence parameters. The use of this facility permits us to combine and corroborate the relationship between the plant physiological traits and the variations in hormone pathways and to identify those metabolites related to these processes. As example, new information about the involvement between photosynthesis, carbon metabolism and changes of cytokinin levels in Arabidopsis was found.

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Polymer solid-phase extraction monolith modified with gold nanoparticles to pre-concentrate biomolecules

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A methodology to synthesize functionalized monoliths with Gold Nanoparticles (AuNPs) and its employ as solid-phase extraction sorbent for pre-concentration of proteins has been developed. For this purpose, glycidyl methacrylate-co-ethylene dimethacrylate polymer was used as powder material and subsequently modified with ammonia and posterior attachment of AuNPs. The resulting material was placed in an SPE cartridge. The influence of several variables (sorbent amount, eluent composition, among others) on SPE extraction was investigated. To perform this study, BSA and Cytochrome C with different pI values (4.7 and 10, respectively), were chosen as probes solutes. Protein recovery was estimated using Bradford assay. The mean recoveries were between 80 and 99.7%. The loading capacity of sorbent and its reusability were also established. Thus, the results showed that the cartridge can be reused for ca. 20 extraction with negligible variations on the sorbent capacity. The practical applicability of this sorbent was demonstrated by processing of vegetal samples for isolation of proteins and satisfactory results were obtained by assay with SDS-PAGE. Then, the present methodology represents a fast and simple pretreatment of complex sample with small amounts of proteins.

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