Proteome analysis in globe artichoke leaves: detection of low abundance proteins and UV-C stress response

Aim of the study

Globe artichoke, besides its role in human nutrition, represents a promising source of biopharmaceuticals. Many of its properties rely on specific metabolites known to be triggered by UV-C irradiation. The main objectives of the project were:

1) To set up an improved protein extraction protocol to study leaf artichoke proteome under stress conditions.
2) To investigate the effect of UV-C light on low abundance proteins, (PEG fractionation protocol).

Analyse

Globe artichoke whole leaf proteome and PEG protein fractions of globe artichoke leaf proteome.

Methodology

Two-dimensional electrophoresis coupled to mass spectrometry. This is one of the most effective approaches for separating and identifying protein extracted from a tissue and can be applied to examine altered expression patterns, comparing protein abundances of related samples, such as mutant and wild type, control and diseased, or following the application of biotic or abiotic stresses.

Plant proteins are typically more difficult to resolve by 2-DE than those from other organisms, because of the presence of many interfering compounds, thus it was mandatory to set out a reliable protein extraction protocol for globe artichoke leaf proteins.

System

Globe artichoke (Cynara cardunculus L. var. scolymus)

Customer

The study was performed in collaboration with DIvAppA, Plant Genetics and Breeding – Università degli Studi di Torino , Centro Ricerche Casaccia, Unità Tecnica BIORAD-FARM, Rome, Plant Research International, Wageningen, The Netherlands

Results

A reliable protein extraction method based on Mg/NP-40 buffer has been established with the goal of eliminating plant interfering compounds, allowing the production of a high resolution 2DE map of globe artichoke whole leaf proteome.

A standardized and reproducible PEG fractionation procedure was then developed to provide an enrichment in low-abundance proteins of globe artichoke leaf proteome. The method allowed the exclusion of highly abundant proteins (such as RuBisCO), and the detection and identification of a large number of proteins.

The PEG fractionation procedure has been adopted to study artichoke PEG -fractionated proteome variation 24h after UV-C irradiation, corresponding to the time of major artichoke healthy secondary metabolites accumulation. PEG fractions were analysed by 2DE coupled with MS/MS analysis. The UV-C responsive PEG-fractionated proteins were identified against a non redundant customised Compositae database, categorised according to Gene Ontology (GO) using Blast2GO v.2.3.6 bioinformatic tool (www.blast2go.org) and visualised on the corresponding pathways by MapMan software.

An overview of the protein variation referred to cellular compartments and biochemical pathways was carried out. A predicted protein interaction network was established and highly connected hub-like proteins were highlighted.

Advantage of the methodology

Proteomic tools have been applied for the first time to assess the modulation of globe artichoke defence mechanisms against UV-C stress. The application of PEG-fractionation allowed the emergence of ‘low-abundance proteins’, undetectable in the total protein extract, representing a huge amount (~5-fold more) of the so-called “hidden proteome”. This finding has also implications for the design of detection strategies based on proteome contrasts on other plant species.

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