Plant Science 195 (2012) 1–9

Contents lists available at SciVerse ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Dynamic proteomic profile of potato tuber during its *in vitro* development

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ARTICLE INFO

Article history:
Received 2 September 2011
Received in revised form 14 June 2012
Accepted 15 June 2012
Available online 21 June 2012

Keywords:
Potato tuber
Tuberization
Stage-specific protein
Shotgun proteomics

ABSTRACT

Potato tuberization is a complicated biochemical process, which is dependent on external environmental factors. Tuber development in potato consists of a series of biochemical and morphological processes at the stolon tip. Signal transduction proteins are involved in the source-sink transition during potato tuberization. In the present study, we examined protein profiles under *in vitro* tuber-inducing conditions using a shotgun proteomic approach involving denaturing gel electrophoresis and liquid chromatography–mass spectrometry. A total of 251 proteins were identified and classified into 9 groups according to distinctive expression patterns during the tuberization stage. Stolon stage-specific proteins were primarily involved in the photosynthetic machinery. Proteins specific to the initial tuber stage included patatin. Proteins specific to the developing tuber stage included 6-fructokinase, phytoalexin-deficient 4-1, metallothionein II-like protein, and malate dehydrogenase. Novel stage-specific proteins identified during *in vitro* tuberization were ferredoxin–NADP reductase, 34 kDa porin, aquaporin, calmodulin, ripening-regulated protein, and starch synthase. Superoxide dismutase, dehydroascorbate reductase, and catalase I were most abundantly expressed in the stolon; however, the enzyme activities of these proteins were most activated at the initial tuber. The present shotgun proteomic study provides insights into the proteins that show altered expression during *in vitro* potato tuberization.

1. Introduction

Potato is ranked the fourth most popular crop worldwide. Potato tubers as the main storage organ have been used for as an alternative food, an animal feed, and as an industrial source for starch and alcohol production. Potato tubers are developed from underground stems that are formed from thickened stolons [1]. Overall, tuberization includes the serial morphological changes of stolon formation, initial tuber development, and tuber maturation, *via* complicated biochemical processes. Despite the physiological and genetic studies of potato tuberization, an in-depth understanding of the global proteome is lacking. In a previous study, the comparative proteomics of potato tuberization identified nearly 100 proteins from the stolon to tuber stages, in which reactive oxygen species-catabolizing enzymes were activated at the initial tuber stage with functionally diverse proteins [2].

Potato tuberization starts with the formation of stolon followed by induction of initial tuber and its development. Each step of the tuberization process is characterized by a change in the expression profiles of specific proteins that regulate the tuber formation. Besides, several external cues and internal factors also influence the tuberization making it a complex biochemical process. The main external cue affecting tuberization is the photoperiod. In general, the biochemical processes of tuber formation involve resource metabolism and its regulation [3]. Phytochrome B can perceive the photoperiod in the leaf, leading to the initiation of tuberization in

Abbreviations: 2DE, 2-dimensional electrophoresis; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; LC–MS/MS, liquid chromatography–mass spectrometry; MudPIT, multidimensional protein identification technology; RT-PCR, reverse transcription polymerase chain reaction; OEC, oxygen-evolving complex; 3-PGA, 3-phospho-D-glycerate; RbcL, ribulose bisphosphate carboxylase/oxygenase large subunit; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; NADPH, nicotinamide adenine dinucleotide phosphate oxidase; SOD, superoxide dismutase; DHAR, dehydroascorbate reductase; APx, ascorbate peroxidase; ROS, reactive oxygen species.

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http://dx.doi.org/10.1016/j.plantsci.2012.06.007

the sub apical tip of the stolon [1]. It is well known that gibberellins act at the tip of the stolon, resulting in the morphological transition into the tuber [4]. Recently, genome-wide transcriptomic analysis revealed more than 1300 genes involved in tuber development [5]; however, details of protein-level regulation remain unclear.

In the present study, an alternative *in vitro* tuberization model system was applied to identify the large-scale proteome using a shotgun proteomic approach involving sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and liquid chromatography–mass spectrometry (LC–MS/MS). The study was aimed to screen the changes in expression profiles of proteins during the tuberization process that could eventually lead us find their putative roles in tuberization. Distinct expression patterns of 251 stage-specific identified proteins in the shotgun proteomics study showed the dynamic changes in their expression at different growth stages. This *in vitro* proteomic study of potato tuber development in comparison with *in vivo* tuberization will prove valuable in constructing an overview of the developmental mechanism of the storage sink organ in potato.

2. Results and discussion

2.1. Global proteomic profiles during potato tuberization

Evaluation of potato cultivars in the field or in greenhouse requires considerable space and time for sufficient tuber development. On the other hand, the application of *in vitro* tuberization has advantages of smaller space and relatively short time for obtaining developed potato tubers. Microtubers grown *in vitro* are known to possess similar characteristics in the accumulation of major storage protein, patatin, as do field-cultivated potato tubers [6,7]. Thus, a proteomic study of potato tuberization *in vitro* can provide a comprehensive understanding of the development of the storage organ, equivalent to the *in vivo* tuber. In this study, we used a shotgun proteomic approach to analyze tuber tissues at specific stages. The process of potato tuberization is generally divided into four main stages; stolon (S0), initial tuber (S1), developing tuber (S2) and mature/big tubers (S3). In this study, tissues for each developmental stage (S0, S1 and S2) were sampled on the basis of their average weight for the corresponding stages (data not shown). The stage S3 was omitted in the present study as at this stage all the biochemical processes associated with tuber development will stop and deposition of storage proteins and storage carbohydrates completes. A non-gel shotgun approach is more efficient than 2DE proteomics, in terms of protein identification and analysis time, to study alterations in the proteomic profile [8].

Potato tuber proteins expressed at specific tuberization stages were analyzed by SDS-PAGE/LC–MS/MS as depicted in Fig. 1A. The LC–MS/MS shotgun proteomics approach combined with gel-based protein separation gives considerable throughput, comparable to the shotgun multidimensional protein identification technology (MudPIT) method, and is a reliable and convenient approach to global proteome identification [9]. The entire set of potato proteins obtained at the stolon (S0), initial tuber (S1), and developing tuber (S2) stages were resolved on 12% SDS-PAGE. Subsequently, 10 excised gel bands were subjected to tryptic digestion prior to LC–MS/MS analysis (Fig. 1B). Using the ion trap mass spectrometer, total peptides in the range of 577–999 Da were acquired from the first and second runs of the large MS/MS spectra (a total of 25,488 and 25,988 at S0; 25,894 and 24,024 at S1; and 25,427 and 22,515 spectra at S2). The final filtered peptides were successfully assigned to 318 and 323 (S0), 261 and 283 (S1), and 293 and 324 (S2)

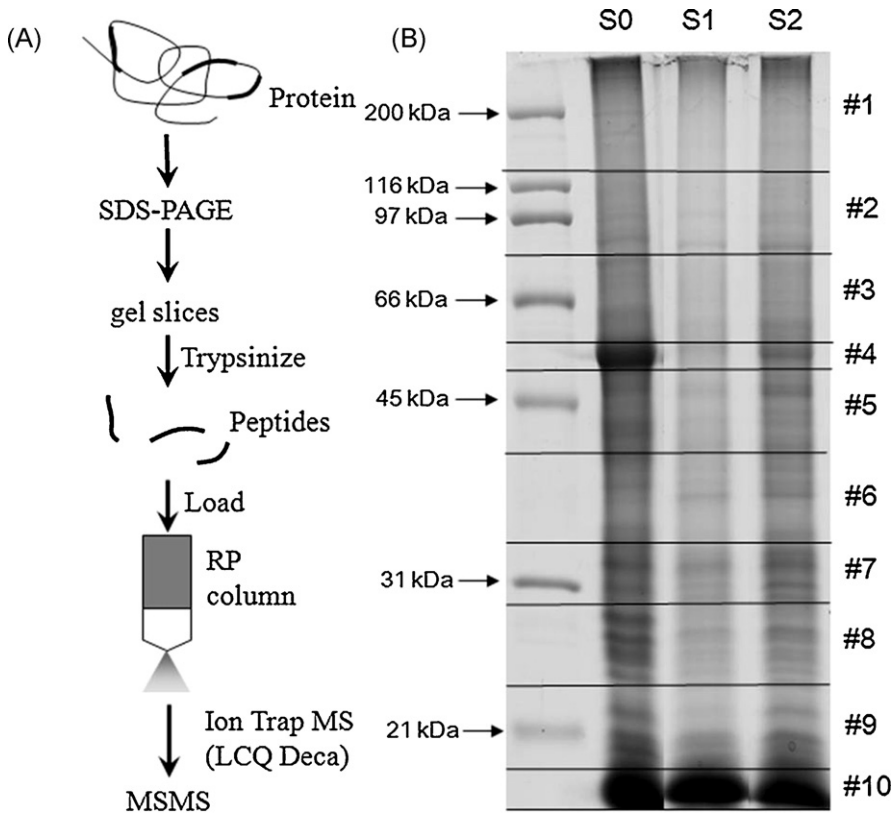

Fig. 1. Scheme of shotgun proteomics approach and SDS-PAGE of potato tuber proteins. (A) Gel-based shotgun proteomic analysis involved the separation of proteins on SDS-PAGE, in-gel trypsin digestion, purification of tryptic digests, and electrospray ionization after reverse-phase column separation. (B) The proteins expressed at specific tuberization stages were separated on a 12% (w/w) SDS-PAGE and stained with CBB R250. Gels were fractionated into 10 parts according to molecular weight prior to LC–MS/MS analysis.