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Comparative metabolite and genome analysis of tuber-bearing potato species

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ABSTRACT

The cultivated potato *Solanum tuberosum* is unrivalled among crop plants for its wild relatives, which potentially represent an important source of genetic diversity to improve the nutritional value of potato varieties and understand metabolism regulation. The main aim of this research was to profile human health-related metabolites in a number of clones from 13 *Solanum* species. Results from HPLC-DAD and LC-ESI-MS analyses highlighted a high interspecific variability in the level of metabolites analysed. Ascorbic acid was confirmed to be the most abundant antioxidant in potato and chlorogenic acid the primary polyphenol. Generally, metabolite-based hierarchical clustering (HCL) and correlation networks did not group clones of identical species in the same cluster. This might be due to various factors, including the outcrossing nature of potato species, gene expression level and metabolic profiling techniques. Access to the genome sequence of *S. tuberosum* and *S. commersonii* allowed comparison of the genes involved in ascorbic acid, aromatic amino acid, phenylpropanoid and glycoalkaloid biosynthesis and helped interpret their respective pathways.

1. Introduction

Potatoes contain good quantities of basic nutrients such as carbohydrates, fibre, and vitamins C and B1, as well as minerals such as potassium and phosphorus. They also have a range of specialized metabolites that play an important role in several processes (Ezekiel et al., 2013). Whereas the phytochemical content of the potato cultivated worldwide (*Solanum tuberosum*) has been extensively studied, few scholars have focused on wild potato species, whose evolutionary diversity is particularly important in both extending knowledge of potato metabolite richness and enhancing its nutritional value.

About 200 potato species grow from the southwestern United States to southern Chile, thus covering a wide ecogeographical range (Machida-Hirano, 2015). They are thought to have a broader genetic base for metabolite content compared to domesticated *S. tuberosum*, whose genetic diversity has been narrowed by human selection. Indeed, metabolic profiling of potato species is an important prerequisite to design appropriate breeding strategies for potato biofortification (Bradshaw et al., 2006). Once identified, selected species can be used as parents in breeding programmes for metabolite enhancement. Fortunately, in potato a vast array of breeding designs and genomic tools is available to achieve this goal (Bradshaw et al., 2006). An additional reason for such an approach results from public concerns over the use of metabolic engineering, which hampers the chances of transgenic plants being commercialized in certain countries. In spite of the natural variation existing in Solanums, the exploitation of wild potato species has been limited in terms of number and breeding objectives (Bradeen and Kole, 2011). Knowledge of the metabolic composition of wild potato relatives is particularly scant. Hence their use to improve the metabolite content of potato varieties and for natural chemical products is very limited. Overall, this contrasts with the fact that several metabolites have beneficial effects on human health and

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may promote plant defence mechanisms as well as fitness (Bourgaud et al., 2001).

Thanks to technological advances in genomics, transcriptomics and metabolomics, exploitation of wild species may be effectively supported by the increasing number of molecular markers and DNA sequence data available as well as the knowledge of genes that contribute to diversification in plant metabolism. Among technological advances, statistical and bioinformatic tools allow all kinds of genome sequence and biological data to be modelled and analysed. In this context, potato may represent a model given the availability of the genome sequence of both cultivated *S. tuberosum* and an interesting wild relative included in this study, *S. commersonii* (Aversano et al., 2015; The Potato Sequencing Consortium, 2011). Such genome sequence information could be useful to identify novel alleles and genes absent in cultivated potato and may help explain *Solanum* species diversity, elucidating the differences in biochemical and molecular mechanisms leading to the formation of various specialized metabolites produced by wild and cultivated species (Hirsch et al., 2016; Machida-Hirano, 2015).

In this work, we report the results of metabolic HPLC-DAD and LC-ESI-MS analysis performed on tuber extracts from 31 clones of 13 *Solanum* species and from cultivated *S. tuberosum*, in order to investigate inter- and intra-specific differences in some human health-related metabolites that represent important breeding targets. Moreover, thanks to the availability of the genome sequence of *S. tuberosum* and *S. commersonii*, we carried out comparative genome analyses to verify whether differences in the metabolite levels were caused by variations in the abundance of genes involved in various metabolic processes. All the data were integrated and subjected to a series of bioinformatics approaches to unravel the main metabolic discriminants responsible for species diversity and to attempt species classification through the use of metabolic data.

2. Results

2.1. Qualitative and quantitative analysis of metabolite content

2.1.1. Targeted analyses of ascorbic acid, tryptophan, tyrosine and specialized metabolites

Metabolites measured in tubers of all species under study were ascorbic acid, free-form tryptophan and tyrosine (Table 1). Furthermore, we detected a series of specialized metabolites (Table 2), mainly phenylpropanoids, comprising caffeic acid (ranging from 2 mg kg^{-1} in ACL1A to 81 mg kg^{-1} in HOU1A), 3-*O*-caffeoyl-5-*O*-feruloylquinic acid (detected in few species), chlorogenic acid (from 2 mg kg^{-1} in ACL1A and STO1A to 2003 mg kg^{-1} in PNT08), cinnamic acid (from roughly 0 in a large group of clones to 6 mg kg^{-1} in HOU1A), coumaric acid (from 1 mg kg^{-1} in BLB2C and FEN1E to 24 mg kg^{-1} in PNT08), ferulic acid (from traces in BLB2C, P1.22, CMM6.6 and PT29 to 57 mg kg^{-1} in ACL2B) and neochlorogenic acid (from traces in ACL2A and ACL3E to 346 mg kg^{-1} in CMM6-6). A second group of phenylpropanoids, including an amide (ferulic acid amide), a conjugated polyamine (caffeoyl spermine) and a flavonoid (rutin), were detected only in a few specific clones. Together with cultivated potato (*S. tuberosum* variety Spunta), ACL2A, ACL3D and PNT04 were the clones in which all the metabolites (sometimes in traces) were found. In the other clones some metabolites were not detected, e.g. tryptophan in ACL1A, caffeoyl spermine in BLB1C and rutin in CMM1T. Compared to Spunta, statistically significant higher levels of metabolites were found in many genotypes, namely ACL3D, CAN1B and CPH1C for ascorbic acid content; ACL1B, ACL2A and HOU1A for free-form tryptophan; and ACL1E and CMM6-6 for free-form tyrosine. The presence of more than one clone belonging to the same species allowed us to highlight significant variability in metabolite content both inter- and intra-species. In *S. acaule,* for example, tyrosine content ranged from 38 mg kg^{1} (ACL 3E) to 229 mg kg^{-1} (ACL1E), with a 6-fold difference between the genotype with the highest and lowest contents. Similarly, in *S. fendleri* neochlorogenic acid content showed a 15-fold difference between FEN1E and FEN1B (12 and 169 mg kg^{1}, respectively). Besides phenylpropanoids, we also measured the two best-known and ubiquitous glycoalkaloids (GAs), α-chaconine and α-solanine (Table 2). As expected, cultivated potato (cv. Spunta) displayed the lowest content of both glycoalkaloids (90 mg kg^{-1} of α-chaconine and 60 mg kg^{-1} of α-solanine). In all genotypes of the other species, glycoalkaloid content varied widely, with HOU1A displaying the greatest amount (18,563 mg kg^{-1} of α-chaconine and 1929 mg kg^{-1} of α-solanine) and CMM1T exhibiting the lowest levels (184 and 136 mg kg^{-1} of α-chaconine and α-solanine, respectively). With regard to the comparison of metabolite content of the two species with available genome sequences (*S. tuberosum* and *S. commersonii*), differences were detected in the concentration of several metabolites, such as ascorbic acid (805 mg kg^{-1} vs. 384 mg kg^{-1}, respectively), free-form tyrosine (199 mg kg^{-1} vs. 54 mg kg^{-1}, respectively) and chlorogenic acid (722 mg kg^{-1} vs. 133 mg kg^{-1}, respectively).

2.1.2. Analysis of phenylpropanoids by UV spectrum and MS identification of metabolites

The results of the analyses carried out are summarized in Figs. 1 and 2, and Supplemental Tables 1 and 2 LC-DAD was exploited to detect tuber metabolites displaying UV maximum absorbance at 254 nm and 320 nm, typical for several phenylpropanoids (Matsuda et al., 2005). Overall, we identified 51 peaks at 254 nm and 33 at 320 nm. In terms of total contents (Fig. 1), we observed a large extent of intra- and inter-specific variations. Compared to *S. tuberosum* (Spunta), eight clones (TAR2B, PNY08, HOU1A, CMM6.6, CMM1T, CHC1A, CHC1B, ACL2B) exhibited a higher content of total phenylpropanoids. The most intense peaks were conserved among the different species, namely peaks 1, 16 and 17 at 254 nm, and peaks 1, 2, 9 and 12 at 320 nm (Fig. 2A and B). Notably, a series of clone-specific peaks were also revealed: for instance, peak 11 at 254 nm only detected in ACL3E, or peak 33 at 320 nm only found in ACL1B. Hierarchical clustering (HCL) was implemented to investigate relationships at phenylpropanoid level (Fig. 2A and B). At 254 nm, we observed conserved clusters among many clones belonging to the same species. A more variable output was obtained in the HCL of 320 nm peaks. As far as the metabolite identification by LC–ESI-QTOF-MS and MS/MS, results are reported in Supplemental Table 3. Fifteen compounds, including ascorbic acid, free-form tryptophan and tyrosine, phenylpropanoids and glycoalkaloids (α-chaconine and α-solanine) were identified from the different dried tubers of wild and cultivated potato species. The molecular formula of the compounds is summarized in Supplemental Table 3.

2.2. Statistical analysis of targeted metabolites

Accumulation patterns of detected metabolites were used to build a dendrogram (Supplemental Fig. 1) that divided our clones into two main clusters based on their metabolic-based similarity. Most of the clones (22) were grouped in one cluster, exceptions being FEN1B, FEN1E, FEN2B, FEN2C, HOU1A, MLT1A, P1.22 and STO1A. Interestingly, the latter genotypes were those with the highest content in α-chaconine compared to the others (Table 2 and Supplemental Fig. 1). To investigate metabolite fluctuations further, we evaluated the possibility of adapting correlation network analysis, considering, for each clone, its resultant metabolic profile (Fig. 3). Interestingly, some species, such as *S. acaule* and *S. fendleri*,

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