Metabolomics (2018) 14:138
https://doi.org/10.1007/s11306-018-1428-7

ORIGINAL ARTICLE

Metabolic diversity in tuber tissues of native Chiloé potatoes and commercial cultivars of *Solanum tuberosum* ssp. *tuberosum* L.

Claudio Inostroza-Blancheteau[1,2] · Franklin Magnum de Oliveira Silva[3] · Fabiola Durán[2] · Jaime Solano[2] · Toshihiro Obata[4] · Mariana Machado[3] · Alisdair R. Fernie[4] · Marjorie Reyes-Díaz[5,6] · Adriano Nunes-Nesi[3]

Received: 21 December 2017 / Accepted: 8 September 2018 / Published online: 4 October 2018
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Abstract

Introduction The native potatoes (*Solanum tuberosum* ssp. *tuberosum* L.) cultivated on Chiloé Island in southern Chile have great variability in terms of tuber shape, size, color and flavor. These traits have been preserved throughout generations due to the geographical position of Chiloé, as well as the different uses given by local farmers.

Objectives The present study aimed to investigate the diversity of metabolites in skin and pulp tissues of eleven native accessions of potatoes from Chile, and evaluate the metabolite associations between tuber tissues.

Methods For a deeper characterization of these accessions, we performed a comprehensive metabolic study in skin and pulp tissues of tubers, 3 months after harvesting. Specific targeted quantification of metabolites using 96 well microplates, and high-performance liquid chromatography combined with non-targeted metabolite profiling by gas chromatography time-of-flight mass spectrometry were used in this study.

Results We observed differential levels of antioxidant activity and phenolic compounds between skin and pulp compared to a common commercial cultivar (Desireé). In addition, we uncovered considerable metabolite variability between different tuber tissues and between native potatoes. Network correlation analysis revealed different metabolite associations among tuber tissues that indicate distinct associations between primary metabolite and anthocyanin levels, and antioxidant activity in skin and pulp tissues. Moreover, multivariate analysis lead to the grouping of native and commercial cultivars based on metabolites from both skin and pulp tissues.

Conclusions As well as providing important information to potato producers and breeding programs on the levels of health relevant phytochemicals and other abundant metabolites such as starch, proteins and amino acids, this study highlights the associations of different metabolites in tuber skins and pulp, indicating the need for distinct strategies for metabolic engineering in these tissues. Furthermore, this study shows that native Chilean potato accessions have great potential as a natural source of phytochemicals.

Keywords Genetic variation · Native potatoes · Exotic germplasms · Metabolite profiling

1 Introduction

The potato (*Solanum tuberosum* ssp. *tuberosum* L.) is currently one of the four most important crops worldwide and is a staple food in the diet of much of the global population (Singh and Saldana 2011). In addition, the Solanaceae family contains many other species that are relevant for human nutrition and health such as tomato, pepper and eggplant. The genus *Solanum* is composed of wild and cultivated types of potato and tomato, and contains over 1000 species. Wild potatoes are native to America, where they display a wide geographical and ecological distribution (Camandro et al. 2012). The potato is extensively grown in southern Chile,

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11306-018-1428-7) contains supplementary material, which is available to authorized users.

✉ Claudio Inostroza-Blancheteau
claudio.inostroza@uct.cl

✉ Adriano Nunes-Nesi
nunesnesi@ufv.br

Extended author information available on the last page of the article

which is considered a sub-center of the origin of the cultivated varieties of this species (Spooner et al. 2005). The native potatoes from Chiloé Island are diverse in size, form, color and flavor, and have been preserved due to the geographical position and the different uses given by farmers (Solano et al. 2013) (Fig. 1). Furthermore, due to its natural conditions and isolation, in Chiloé Island, a great number of native potato accessions have proliferated. Between these accessions, there is substantial variety in quality and suitability for cultivation at different periods in the farming calendar, and different forms of preparation and consumption (Contreras et al. 1981). Hence, this collection represents a rich source of exotic germplasm.

Native potatoes in general are rich in phytochemicals such as polyphenolic compounds, flavonoids, anthocyanins, vitamin C and carotenoids (Reyes et al. 2005; Lachman et al. 2009). These metabolites have high free-radical scavenging activity and may help protect proteins, lipids and DNA against reactive oxygen species, which are implicated in age-related neuronal degeneration and many chronic diseases (Ames et al. 1993; Teow et al. 2007). Moreover, extracts derived from tuber skin possess strong antioxidant activity in in vitro models (Singh and Rajini 2004) and are able to protect erythrocytes against oxidative damage (Singh and Rajini 2008). In addition, the presence of phytochemicals such as anthocyanins has been associated with health benefits (Lovat et al. 2016), and many of the most bioactive compounds are located in the skin of the tubers (Scalbert et al. 2005; Albishi et al. 2013; Al-Weshahy et al. 2013). Thus, colored native potatoes are potential sources of natural phytochemical compounds and could thus be used to restore this trait in paler cultivated varieties (Jansen and Flamme 2006; Akyol et al. 2016). Recently, studies aimed at understanding the major metabolic pathways that are active in potato pulp tissues have been performed (Dobson et al. 2008, 2010; Carreno-Quintero et al. 2012). These studies focused on starch content, sugars, glycoalkaloids, and pulp color and quality. Since high natural availability of metabolites was found in pulp and skin of tubers from native potatoes, this set of genotypes represents an important source of genetic variability for breeding programs aimed at improving tuber quality.

Despite its importance for human nutrition and health, it remains unclear how exactly primary and secondary metabolism are regulated and how the final chemical composition of tuber tissues is determined. Here, we used a panel of ten native potatoes from Chiloé Island, and one continental Chile accession with varying tuber sizes, shapes and colors to characterize the metabolite profiles from skin and pulp tissues and investigate their connection with antioxidant activity in tubers. In this study, the importance of these metabolic differences is discussed with respect to the use of native potatoes as a key resource in metabolomics-assisted breeding programs.

2 Materials and methods

2.1 Plant material and growth conditions

We selected ten native accessions of potato (*S. tuberosum* ssp. *tuberosum* L.) from different areas of Chiloé Island, and one accession from Los Muermos (Chilean mainland), for characterization at the metabolic level. The chosen native potatoes were selected according to their skin and pulp color, and the size and shape of the tuber (Fig. 1; Table 1). In addition, we analyzed two improved cultivars, Desirée and Yagana, as controls for red- and white-skinned accessions, respectively (Fig. 1; Table 1).

This collection of native potatoes was clonally-propagated in the same field under similar management, climate and soil conditions, at the Experimental Station of the School of Agronomy at the Universidad Católica de Temuco, in the Araucanía Region, Chile. The morphological traits vary widely according to their genotype; elongated, round, oval, fusiform and elliptical tuber shapes are observed (Fig. 1). Each cultivar was grown in four independent blocks that were randomly located in the same field. Each block contained one hundred and twenty plants from which all tubers were harvested. After harvesting tubers free of mechanical and/or physiological damage, samples were maintained in semi dark conditions at 8 °C. After 3 months of storage, samples were prepared for metabolome profiling by discarding the proximal and distal part of the tuber, and dividing the middle part into skin (epidermis and cork cells) and pulp (medulla and perimedulla cells) tissues, which were sliced, immediately frozen in liquid nitrogen, ground to a fine powder and stored at − 80 °C until analysis.

2.2 Determination of total antioxidant activity and phenolic content

The antioxidant activity of skin and pulp was quantified in methanolic extracts using the free radical 2,2-diphenyl-1-picrylhydrazyl scavenging method as described by Chinnici et al. (2004), with some modifications. The absorbance was measured at 515 nm in a spectrophotometer (Thermo Spectronic, GENESYS 10uv, Rochester, New York, USA) using Trolox (Sigma Aldrich, St Louis, MO, USA) as the standard. The total phenolic (TP) content was determined by the Folin–Ciocalteu method (Slinkard and Singleton 1977) using chlorogenic acid as standard. Skin and pulp samples were extracted with methanol (80% v/v), and the extract was centrifuged at 10,000×*g* for 5 min at 4 °C and the supernatant stored at 4 °C until use. The absorbance was measured

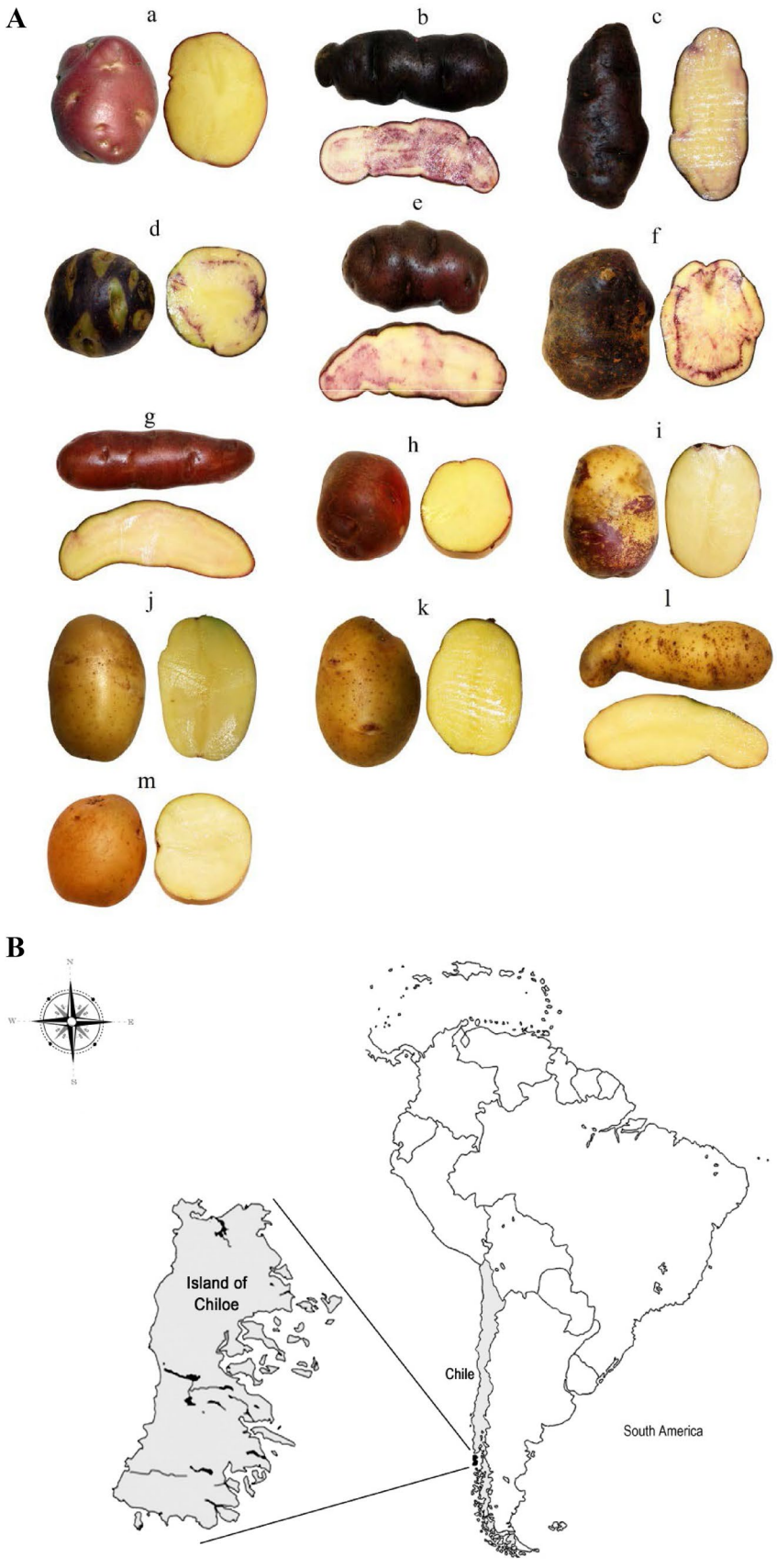


Fig. 1 **A** Diversity in shape and color of native potatoes. (*a*) Desireé, (*b*) Meca de gato morada larga, (*c*) Michuñe azul, (*d*) Guicoña, (*e*) Michuñe negro, (*f*) Cauchau, (*g*) Guadacho colorado, (*h*) Murta, (*i*) Clavela morada, (*j*) Yagana, (*k*) Lengua de vaca, (*l*) Guadacho blanco, (*m*) Tonta. **B** Map of Chiloé Island, Chile

Table 1 Origin and local name of 13 accessions of potato (*Solanum tuberosum* L.)

Accession	Local name	Origin
UCT-6Gc	Guadacho colorado	Chonchi, Chiloé
UCT-24Tn	Tonta	Castro, Chiloé
UCT-22Cm	Clavela morada	Castro, Chiloé
UCT-25Gñ	Guicoña	Quellón, Chiloé
UCT-18Mn	Michuñe negro	Isla grande, Chiloé
UCT-27Mu	Murta	Quellón, Chiloé
UCT-1Ma	Michuñe azul	Isla grande, Chiloé
UCT-2Lv	Lengua	Castro, Chiloé
UCT-8Gb	Guadacho blanco	Ancud, Chiloé
UCT-10MgL	Meca gato morada larga	Los Muermos, Chilean mainland
UCT-35Cau	Cauchao	Isla grande, Chiloé
Desirée	Desirée	Introduced Cultivar ZPC-Holland
Yagana-INIA	Yagana	Chilean Cultivar

at 765 nm and the results expressed in mg of chlorogenic acid equivalents (CAE) per gram fresh weight (FW).

2.3 Determination of total flavonoid and anthocyanin contents

Flavonoids were determined as described by Meyers et al. (2003), with minor modifications. The results are expressed as mg of rutin equivalents per gram FW (mg rutin equivalent g^{-1} FW) as described in Ribera et al. (2010). The absorbance was measured at 515 nm in a spectrophotometer (ThermoSpectronic, GENESYS 10uv, Rochester, New York, USA) using rutin (Sigma Aldrich, St Louis, MO, USA) as standard.

The extraction of anthocyanins was performed by grinding 0.1 g of fresh material in a cold mortar, and adding 1 mL of acidified methanol. After extraction, the anthocyanin levels were determined by spectrophotometry at 530 nm and at 657 nm with a molar extinction coefficient for cyanidin-3-glucoside of 29,600 as previously described (Nyman and Kumpulainen 2001). The total anthocyanin contents are expressed as mg of cyanidin-3-glucoside equivalents (c3g) per gram FW.

2.4 Determination of metabolite levels

The frozen skin and pulp samples were lyophilized, and metabolites extracted twice with 80% ethanol and once with 50% ethanol. Sucrose, fructose and glucose were determined exactly as described previously by Fernie et al. (2001), and total amino acids as described by Cross et al. (2006). In the insoluble fraction, the starch and total protein contents were determined as indicated by Cross et al. (2006).

For metabolite analysis by gas chromatography-time of flight-mass spectrometry (GC-TOF-MS), the extraction of metabolites from lyophilized samples was performed as described by Lisec et al. (2006). Metabolite derivatization, standard addition, and sample injection for GC-MS were performed as previously described (Osorio et al. 2012). Chromatograms and mass spectra were evaluated using Chroma TOF 1.0 (Leco, http://www.leco.com/) and TAGFINDER 4.0 software (Luedemann et al. 2008). The mass spectra were cross-referenced with those in the Golm Metabolome Database (Kopka et al. 2005). The amount of each metabolite obtained by GC-TOF-MS was determined as relative metabolite abundance, calculated by normalization of signal intensity to that of ribitol, which was added as an internal standard, and then expressed per dry weight (DW) of the material, exactly as previously described (Lisec et al. 2006).

2.5 Statistical analyses

Statistical analyses were performed using the GENES program (Cruz 2013) and R statistical software (http://www.r-project.org). All data were subjected to a one-way analysis of variance (ANOVA), and the means were tested by the Tukey test at a 5% significance level. In order to reduce the dimensionality of the data set, multivariate analysis by Principal Component Analysis (PCA) was performed with Minitab® 17 (Minitab 17 Statistical Software). For PCA, data were normalized to maximize the variance of each component. Furthermore, a graphical representation of the metabolic profiling data set was shown as a heatmap using Multiple Experiment Viewer Software (MeV) version 4.5 (Saeed et al. 2003). Pearson correlation analysis and correlation networks of phytochemical compounds and selected metabolites were obtained using a *t*-test (5% significance level) to examine the relationships between these variables.

3 Results

3.1 Metabolite levels in skin and pulp tissues of tubers from native potatoes

In order to place the visible differences in tuber skin and pulp from different native potatoes in a metabolic context, we performed a detailed metabolic characterization of these tissues (Figs. 2, 3, Supplementary Table SI). First, we studied the contents of starch, glucose, fructose and sucrose in each genotype. In terms of starch content, we observed more starch in the pulp than in the skin (Fig. 2a, b), particularly in Desireé and Michuñe negro accessions (37.7 and 50.1%

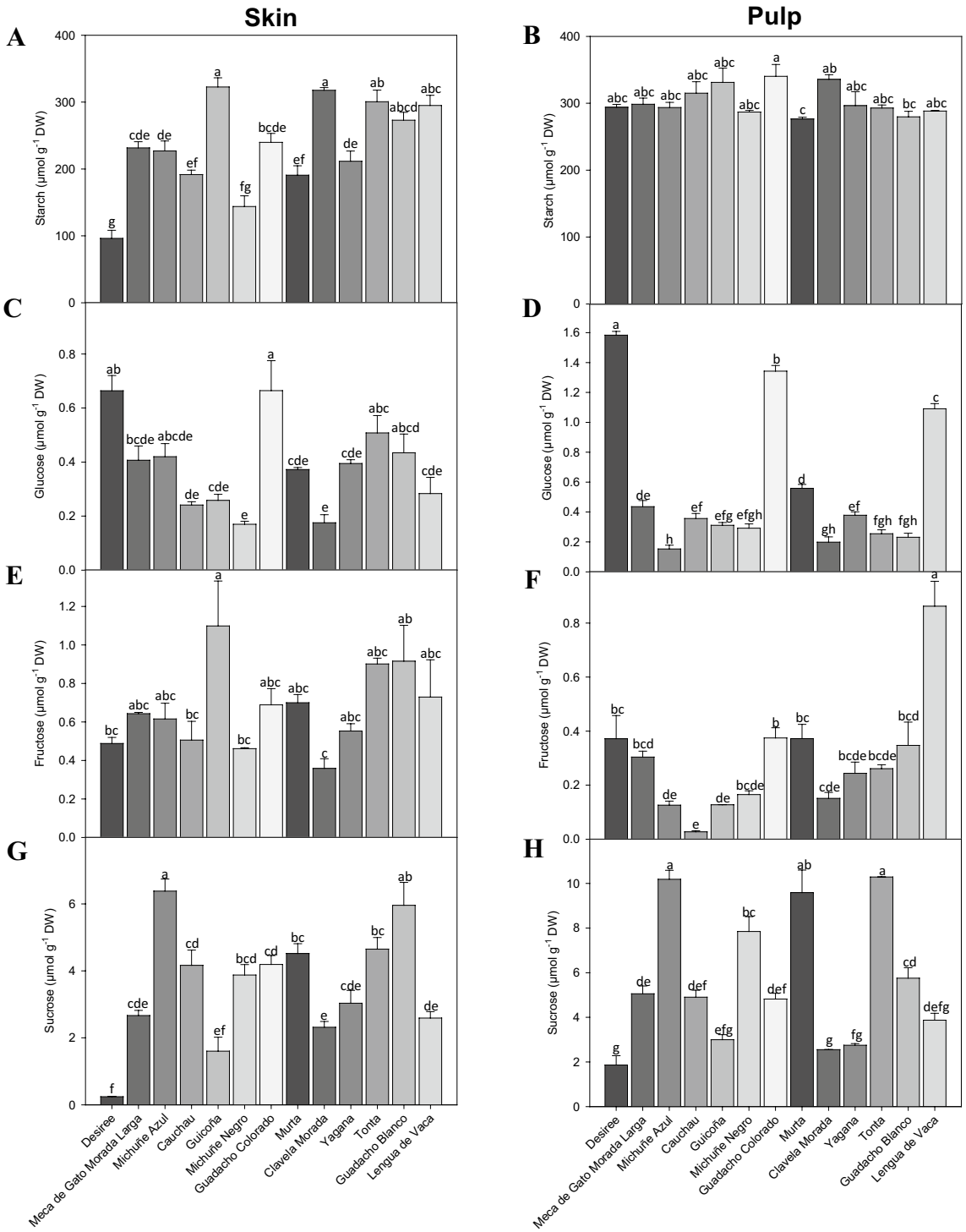


Fig. 2 Carbon metabolism related compounds in skin (**a, c, e, g**) and pulp (**b, d, f, h**) tissues of tubers from 11 Solanum tuberosum ssp. tuberosum L. landraces from Chiloé Island and two commercial cultivars (Desireé and Yagana). **a, b** starch; **c, d** glucose; **e, f** fructose; **g, h** sucrose. Values are presented as mean ± SE (n = 5). Values marked with a different letters were determined by Tukey test to be significantly different ($P < 0.05$) from each other. *FW* fresh weight, *DW* dry weight

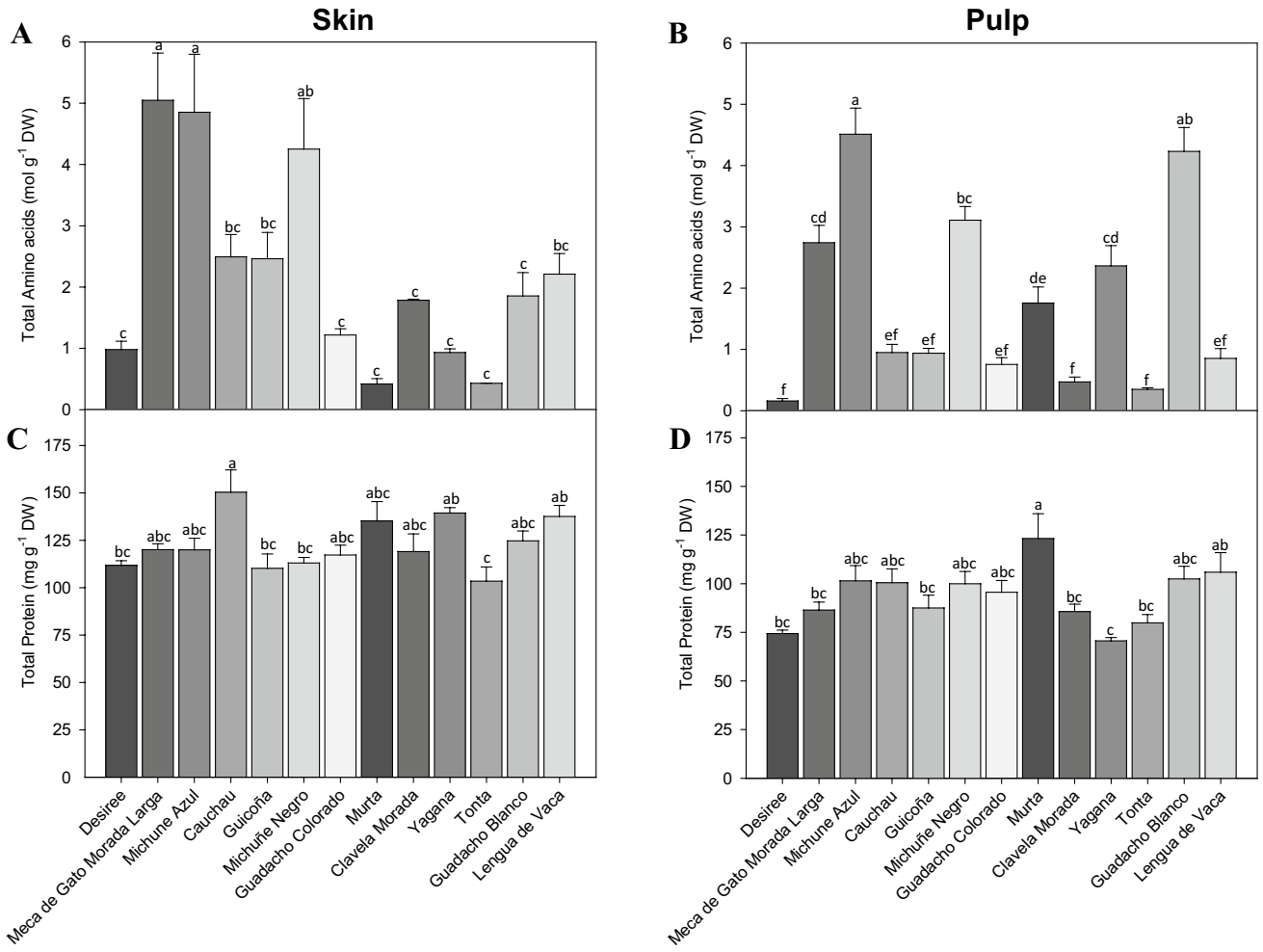


Fig. 3 Nitrogen metabolism compounds in skin (**a, c**) and pulp (**b, d**) tissues of tubers from 11 native potato (*Solanum tuberosum* ssp. *tuberosum* L.) accessions and two commercial cultivars (Desireé and Yagana). **a, b** total amino acids; **c, d** total protein levels. Values are shown as mean ± SE (n = 5). Values marked with a different letters were determined by Tukey test to be significantly different ($P < 0.05$) from each other. *FW* fresh weight, *DW* dry weight

greater in pulp, respectively). On the other hand, the starch levels in the Guicoña, Clavela morada, Tonta, Guadacho blanco and Lengua de vaca, were not significantly different between these tissues (Fig. 2a, b).

High variability was observed between the content of glucose, fructose and sucrose in both tissues (Fig. 2c, h). The highest level of sucrose was observed in pulp of Michuñe azul, Murta and Tonta accessions, with levels that fluctuated between 9 and 11 µmol g^{-1} DW (Fig. 2h). The lowest level of sucrose was observed in skin and pulp from the Desireé cultivar (Fig. 2g, h), which had the highest concentration of glucose in pulp tissue (Fig. 2d). The level of glucose observed in this cultivar was 1.6 µmol g^{-1} DW in the skin and 0.8 µmol g^{-1} DW in the pulp. Similar glucose contents were observed in Lengua de vaca and Guadacho colorado accessions (Fig. 2c, d). The levels of fructose were higher in the skin compared to the pulp tissues in all genotypes analyzed, with the exception of the Lengua de vaca accession (Fig. 2e, f).

With respect to total amino acid levels, substantial variability was observed among genotypes in both tissues (Fig. 3a, b). The Tonta accession had similar levels of amino acids in skin and pulp. The highest levels of total amino acids were found in the skin of Meca de gato morada larga, Michuñe azul and Muchuñe negro (Fig. 3a). Higher protein levels were observed in skin with values varying between 110 and 150 mg g^{-1} DW (Fig. 3c), and greater than those in the pulp (Fig. 3d). However, similar levels were observed among the 13 potato genotypes in both tissues.

3.2 Anthocyanin content and antioxidant activity in skin and pulp tissues of tubers from native potatoes

Since potatoes are rich sources of phenolic compounds such as anthocyanins and phenolic acids, we extended our characterization of the native potatoes to metabolites related to antioxidant activity. Firstly, we evaluated the total anthocyanin content. For these compounds, significant differences were obtained between skin and pulp (Fig. 4a, b). Some of the native accessions, such as Guicoña and Tonta, displayed remarkably high levels of anthocyanin in both tissues (Fig. 4a, b). In the skin of Clavela morada, the total anthocyanin content was around 10-fold greater in skin compared to pulp, while in other accessions the differences were considerably lower (Fig. 4a). The anthocyanin levels varied in skin from 0.1 to 1.8 mg g^{-1} FW and in pulp between 0.1 and 0.3 mg g^{-1} FW (Fig. 4a, b).

The total content of phenolics observed in skin fluctuated between 1 and 3 mg g^{-1} FW, whereas in pulp, levels reached 0.5 mg g^{-1} FW (Fig. 4c, d). High levels of phenolics were observed in the skin of Guadacho colorado and Lengua de vaca (Fig. 4c). The native potatoes Lengua de vaca, Clavela morada, Tonta and Guadacho colorado displayed higher phenolic contents in the skin in comparison with pulp tissue (Fig. 4c, d).

Concerning the total flavonoid levels, skin tissues possessed more than pulp tissues. These values ranged from 3000 to 7600 mg rutin equivalents g^{-1} FW in skin and 190–430 mg rutin equivalents g^{-1} FW in pulp tissues (Fig. 4e, f). High flavonoid levels were observed in the skin of Lengua de vaca, and less in Clavela morada, Guicoña and Michuñe negro, in comparison with Desireé and Yagana (Fig. 4e). In pulp tissues, increased levels were observed only in Meca de gato morada larga and Michuñe azul, compared to both commercial cultivars (Fig. 4f).

Furthermore, we detected differences in antioxidant activity between skin and pulp tissues (Fig. 4g, h). High antioxidant activity was observed in the skin of Lengua de vaca, Michuñe azul, Clavela morada and Tonta in comparison with both commercial cultivars (Fig. 4g). In pulp, higher antioxidant activity was observed in Michuñe azul, Michuñe negro, Murta, Guadacho blanco and Lengua de vaca, and less activity in Cauchau, Guadacho Colorado and Yagana, in comparison with Desireé (Fig. 4h).

3.3 Pearson correlation analysis and integration of primary metabolites and phytochemical traits

To assess the level of association between the main carbon and nitrogen containing compounds and phytochemical traits, we next calculated the Pearson correlation coefficients for all pairs of primary metabolites (protein, total protein, hexoses, sucrose and starch) and phytochemical traits. The full data set of correlation coefficients is presented in a heat map (Fig. 5a, b). In skin tissues, starch content positively correlated with anthocyanin levels, whereas in pulp, starch negatively correlated with anthocyanin (Fig. 5a). Interestingly in pulp tissues, antioxidant activity positively correlated with protein and amino acid levels and negatively correlated with starch levels (Fig. 5b). Furthermore, in skin tissues phenolic content correlated positively with antioxidant activity, and anthocyanin negatively correlated with flavonoids.

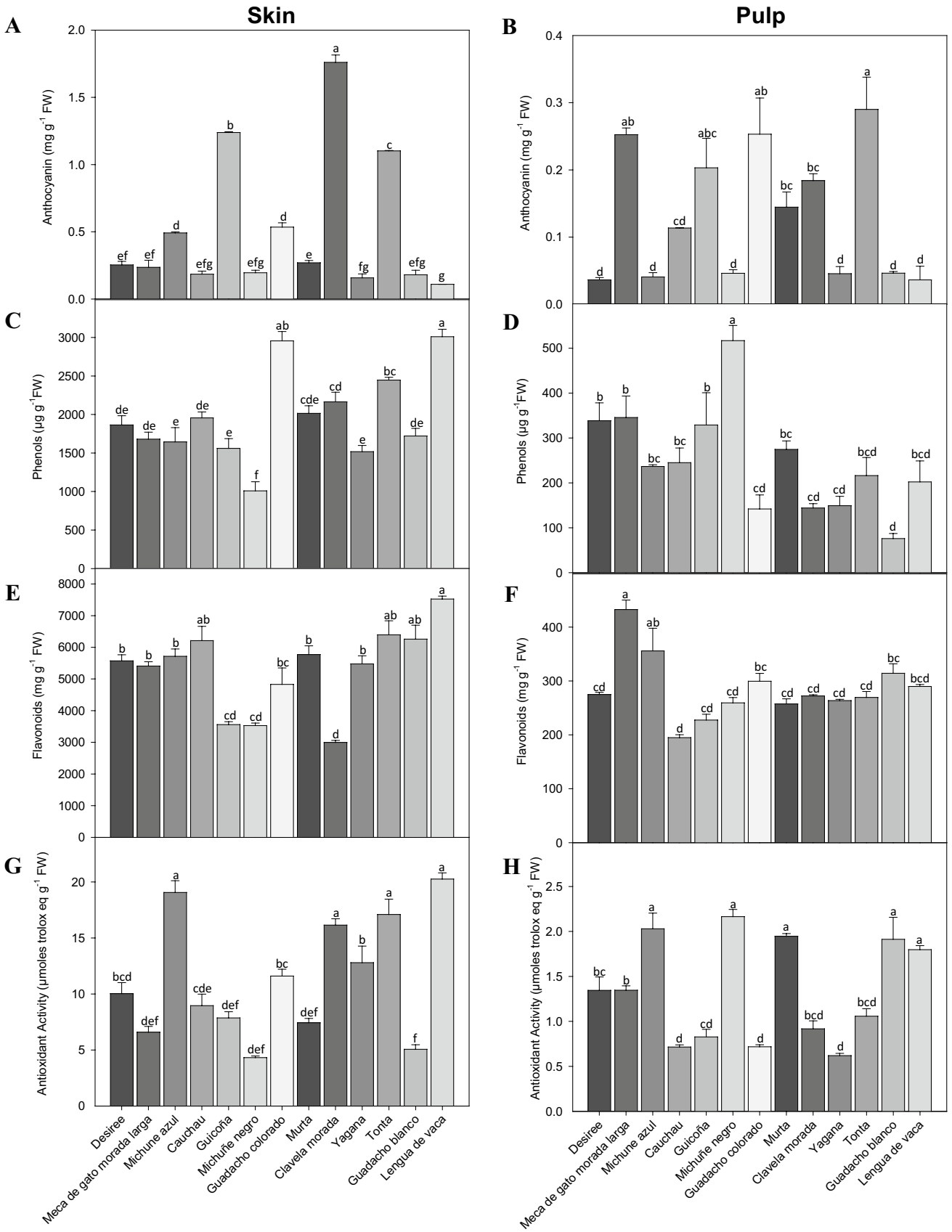
For a better understanding of the association between the evaluated traits of all potato genotypes analyzed, the correlation coefficients were arranged in a correlation network (Fig. 5c, d). In this analysis, a large number of associations was observed. In skin tissue, protein and total amino acids showed a negative correlation with those of anthocyanin and phenolics, respectively (Fig. 5c). On the other hand, in pulp tissue there was a positive correlation between nitrogen metabolism and phytochemicals such as flavonoids and antioxidant activity (Fig. 5d).

When we analyzed the skin variables related to carbon metabolism, we observed that starch levels positively correlated with most of the phytochemicals analyzed. Nonetheless, in pulp tissue this correlation was opposite. Although starch seems to play a central role in the relationship between phytochemicals and carbon metabolism in skin tissue, glucose levels also displayed a noticeable correlation with phytochemicals, such as phenolics and flavonoids.

3.4 Primary metabolite profiles in skin and pulp of tubers from native potatoes

To investigate the metabolites of the major primary pathways in both tuber tissues in a broader metabolic context, we performed metabolic profiling analysis. Metabolite profiles were obtained via the relative quantification of 79 and 73 metabolites of known chemical structure in skin and pulp tissues, respectively (Supplementary Tables SII, SIII). These compounds include most plant amino acids, organic acids and sugars. In order to display a general overview of the results, part of the data set from the metabolite profiling study is presented as a heat map (Fig. 6).

In total eighteen amino acids were analyzed in the skin and pulp tissues of all 13 accessions (Fig. 6). Low variability between the native potatoes, which were significantly different from the commercial cultivars, was found for alanine, asparagine, glycine, histidine and isoleucine in both skin and pulp tissues. Interestingly, the levels of alanine and glycine were significantly lower in skin of the native potatoes in comparison with Desireé and Yagana cultivars.


Skin
Pulp
A
B
C
D
E
F
G
H
Anthocyanin (mg g-1 FW)
Phenols (µg g-1FW)
Flavonoids (mg g-1 FW)
Antioxidant Activity (µmoles trolox eq g-1 FW)
Desiree
Meca de gato morada larga
Michune azul
Cauchau
Guicoña
Michuñe negro
Guadacho colorado
Murta
Clavela morada
Yagana
Tonta
Guadacho blanco
Lengua de vaca

◂**Fig. 4** Secondary metabolites and antioxidant activity in skin (**a, c, e, g**) and pulp (**b, d, f, h**) tissues of tubers from 11 native potato (*Solanum tuberosum* ssp. *tuberosum* L.) accessions and two commercial cultivars (Desireé and Yagana). **a, b** anthocyanin; **c, d** total phenols; **e, f** total flavonoids; **g, h** total antioxidant activity. Values are shown as mean ± SE (n = 5). Values marked with a different letters were determined by Tukey test to be significantly different ($P < 0.05$) from each other. *FW* fresh weight, *DW* dry weight

The levels of tricarboxylic acid cycle intermediates 2-oxoglutarate, citrate and pyruvate in skin, and fumarate, isocitrate and malate in pulp displayed only minor variations among the genotypes (Fig. 6). The levels of succinate did not vary between the genotypes in both skin and pulp tissues. However, the levels of glycerate, maleate and nicotinate varied between genotypes in skin tissues, and were significantly lower in several of the native accessions compared with the Desireé cultivar.

In addition, we evaluated sugars and related compounds in skin and pulp tissues. Of the sugars identified, isomaltose, melibiose, sorbose and xylose were detected only in pulp tissues. Meanwhile, cellobiose, gentiobiose, maltotriose, palatinose and turanose were quantifiable only in the skin tissue (Fig. 6). Surprisingly the levels of cellobiose, maltose, palatinose and turanose did not vary between the native accessions but were reduced in skins of all accessions in comparison with Desireé. In pulp tissues, reduced levels of maltose, sorbose and xylose were observed in most of the studied accessions. Concerning the levels of hexose phosphates, minor variability in the levels of glucose-6-P and fructose-6-P was found in the skin tissue of the different genotypes. In pulp tissues, fructose-6-P was essentially invariant (Fig. 6). In addition, ribulose-5-P levels in skin tissues were significantly lower in seven native potato accessions.

Moreover, several other metabolites related to different pathways were evaluated. In skin tissues, significant differences in the levels of calystegine, 2-hydroxy-cinnamic acid, putrescine and spermine were observed between the native potatoes and Desireé (Supplementary Table II). In pulp tissue significant differences were observed for some of the native potato accessions in the levels of calystegine, myo-inositol, myo-inositol-1-P and 3-caffeoyl-quinic acid in comparison with Desireé (Supplementary Table III).

3.5 HCA and PCA of the metabolite profiles of skin and pulp tissues of native potatoes

To identify the differences between the metabolite profiles of skin and pulp tissues of native potatoes, the metabolite data set were analyzed using PCA (Fig. 7). In skin tissue, the first principal component (PC1) and the second principal component (PC2) accounted for 25.2% and 16.7% of the total variation, respectively (Fig. 7a). On the other hand, in pulp tissue, PC1 and PC2 explained 30.9% and 16.6% of the total variance, respectively (Fig. 7b). When we compared the PCA scores plot of skin tissues, we observed a clear separation in PC1 of a group of nine native potatoes from the cultivar Desireé and three other accessions (Yagana, Cauchau and Guadacho colorado) (Fig. 7a). In pulp tissues, there was a separation of two groups of accessions and Desireé (Fig. 7b). Interestingly, in both tissues, when the clusters are analyzed in detail, it is clear that the isolation of the cultivar Desireé is more related with sugars than amino acid levels, in comparison with the more distant group of native potatoes (eg Michuñe negro, michuñe azul and Meca de gato). Together these data suggest that the most substantial differences in terms of metabolites in native potatoes are found in amino acid and sugar levels. In addition, this result indicates that, in both skin and pulp, the native potato accessions have very similar metabolite profiles between them, but contrast dramatically with the commercial cultivar Desireé.

4 Discussion

4.1 Phytochemical content in native potatoes

Potatoes, specially the purple colored ones, are sources of antioxidant compounds including polyphenols, carotenoids and vitamins, pointing to their relevance not only as a starchy food, but also as a vegetable important for health (Chun et al. 2005; Ezekiel et al. 2013; Chandrasekara and Kumar 2016). Several studies reported high antioxidant activity of potato tubers (Mattila and Hellstron 2007; Lachman et al. 2009), flowers (Sosulski et al. 1982), stems and leaves (Hyon et al. 2008), as well as in tuber skin and pulp (Valcarcel et al. 2015). In this study, we quantified the levels of phenols, flavonols, anthocyanins and the total antioxidant activity (Fig. 4). The levels of these phytochemicals varied among the analyzed potato accessions in both skin and pulp tissues. In general, the highest antioxidant activities were found in purple-skinned and black pulp accessions, like Clavela morada (in skin) and Michuñe azul (in both skin and pulp), and also in the white-skinned and white pulp Lengua de vaca (in both skin and pulp) and Tonta (in skin). In agreement with these results, potato tubers have been described as a rich source of phenolic compounds, with almost 50% of them located in the skin and adjoining tissues (Al-Weshahy et al. 2009; Valcarcel et al. 2015). In our study, the levels of phenolic compounds in skin varied depending on the color and variety of the potato genotypes (Fig. 4). The phenolic compounds are present in the potato skin and flesh; however, the skin is reported to have the highest amounts (Ezekiel et al. 2013). We found particularly high phenolic levels in the skin of Guadacho colorado, Clavela morada, Tonta and Lengua de vaca, mostly associated with high antioxidant activity in these native accessions (Fig. 4). Because of their

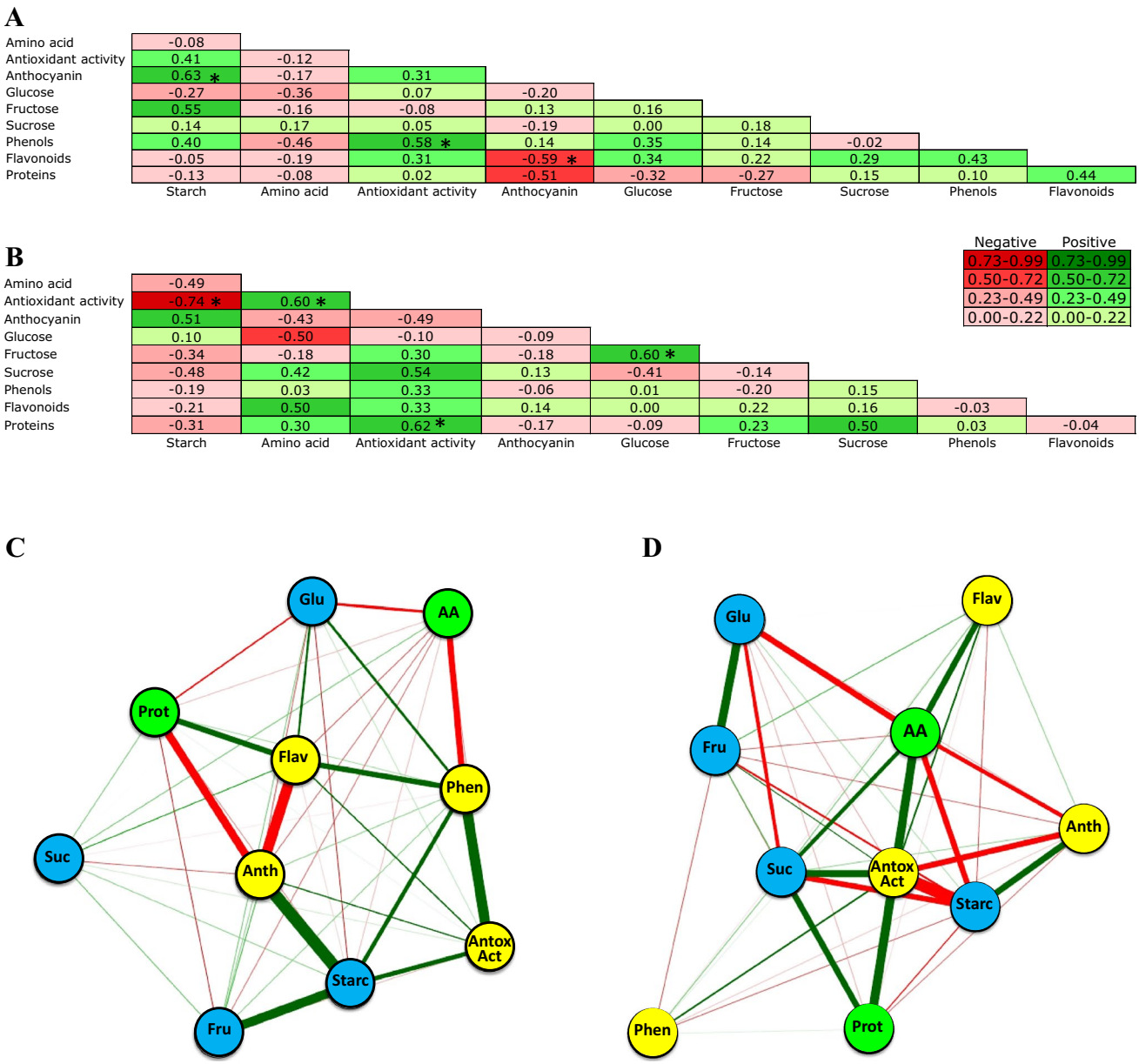


Fig. 5 Correlation and network analysis of phytochemical compounds and other metabolites in skin and pulp of thirteen native potatoes accessions. Correlation matrix based on Pearson coefficients derived from the data obtained from skin (**a**) and pulp (**b**) indicated by color gradient, with positive and negative correlations being distinguished by green and red, respectively. Significant correlation coefficients are indicated by asterisk (*). The correlation network from skin (**c**) and pulp (**d**) with trait associated to carbon metabolism shown as blue dots, nitrogen metabolism as green dots and phytochemical compounds yellow colored. Green connecting lines represent positive correlations and red connecting lines negative, respectively. Line width is proportional to the strength of the correlation. *AA* total amino acids, *Antox Act* antioxidant activity, *Anth* anthocyanin, *Glu* glucose, *Fru* fructose, *Suc* sucrose, *Phen* phenols, *Flav* flavonoids, *Prot* total protein levels

potential health benefits, in the last decade, there has been increasing attention given to new sources of natural antioxidant phytochemicals (Akyol et al. 2016). That way, these cultivars could be an ideal source to produce potato plants with healthier tubers by means of the transgenic or classical genetic approaches.

The Clavela morada accession possesses a speckled skin coloration, probably attributable to regions exhibiting

enrichment of anthocyanins (Fig. 4a). Red and purple skin color of pulp may be partially or entirely due to anthocyanin accumulation (Brown 2005). By contrast, accessions such as Tonta (non-colored skin) and Clavela morada (blue skin) may contain very high levels of phenolic acids and/or other non-anthocyanin flavonoids. Valcarcel et al. (2015) reported that anthocyanins are absent or only present in negligible amounts in the skin of non-colored genotypes. These studies are consistent with the negative correlation observed between flavonoids and anthocyanins in our work (Fig. 5a). Positive correlations were found among total phenolics, total flavonoids and antioxidant activity (Valcarcel et al. 2015). Moreover, our correlation data analyses indicate that phenolic levels in skin tissues are positively correlated with antioxidant activity. Surprisingly, the associations described between these phytochemicals and antioxidant activity were significant in pulp tissues. Together these results indicates that the secondary metabolism taking plance in pulp and skin tissues is quite complex and therefore further research efforts at metabolite and transcript levels should be performed for enhancing our current understanding of the presented associations in tuber tissues of native potatoes.

4.2 Phytochemical compounds and primary metabolites in tuber tissues of native potatoes

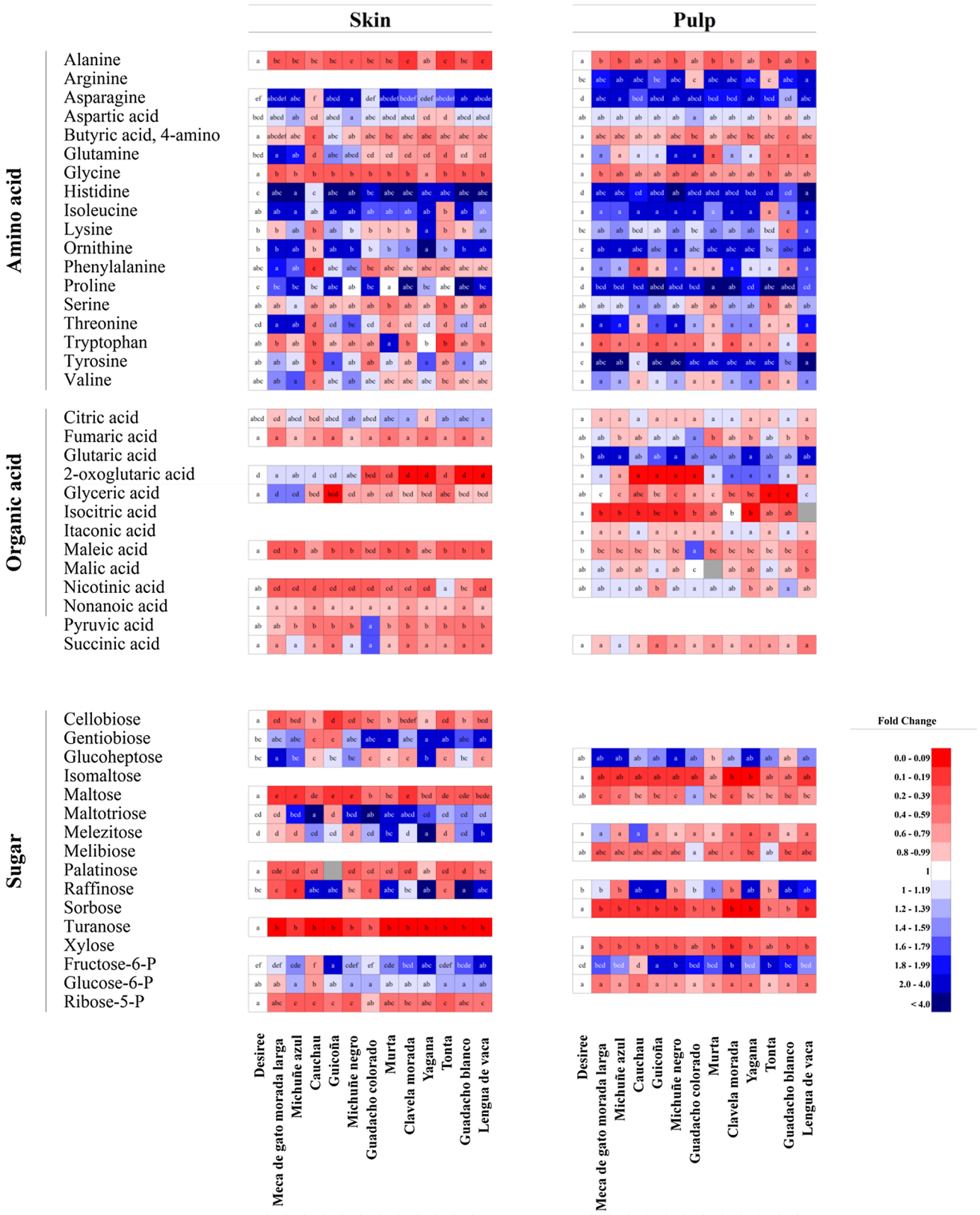
In this study, distinct associations were observed between anthocyanin levels and antioxidant capacity with starch, protein and amino acid contents (Fig. 5). In pulp tissues, antioxidant activity was inversely correlated with starch and positively correlated with amino acid and protein levels. Consistently, changes in antioxidant capacities in tubers of potato plants overexpressing and repressing key enzymes of the flavonoid synthesis pathway such as chalcone synthase, chalcone isomerase and dihydroflavonol reductase, increase the synthesis of phenolic compounds accompanied by a decrease in starch and glucose levels in the transgenic plants (Lukaszewicz et al. 2004). Flavonoids and phenolic compounds may also respond to the carbohydrate level by regulatory mechanisms that efficiently divert carbon towards the biosynthesis of aromatic amino acids, precursors of the phenylpropanoid pathway (Obata and Fernie 2012). Thus, these results suggest that in pulp tissues, the antioxidant activity and synthesis of related compounds is associated with the availability of carbohydrates for phenolic compound synthesis. Interestingly, the results observed in skin suggest a weak correlation between starch and antioxidant activity, and a strong positive correlation between starch and anthocyanin levels, indicating that in skin tissues alternative associations between these metabolites take place. Taken together, our results indicate tight and distinct associations between primary and secondary metabolites in skin and pulp tissues. However, since the levels of phenolics and flavonols, as well as antioxidant activity in potato tubers vary according to the season and field of cultivation (Payyavula et al. 2012; Valcarcel et al. 2015), additional experiments and analysis are needed to fully understand these associations.

4.3 Metabolite markers for breeding programs

In potato tubers, different primary metabolites have been proposed to function as predictors for agronomically important traits such as black spot bruising and chip quality (Steinfath et al. 2010) and starch levels (Carreno-Quintero et al. 2012). In our study, we observed variation in the levels of potential metabolite markers among the studied accessions. For example, glucose and fructose, which are involved in the Maillard reaction, which is responsible for the browning of potato chips during frying and also leads to the accumulation of carcinogenic acrylamide (Zhang and Zhang 2007), were drastically reduced in the pulp of some of the native accessions (Fig. 2d). Furthermore, we were able to quantify valine, serine, threonine, glutamine and tyrosine in both skin and pulp tissues (Fig. 6). A large variation in the levels of these amino acids in skin was observed between native potato accessions. These amino acids are associated with susceptibility of potato tubers to black spot bruising (Steinfath et al. 2010). This physiological disorder reduces the commercial value of the tubers and is characterized by the formation of dark-blue to blackish melanin spots, below the skin (Laerke et al. 2002). Another important metabolite marker that displayed different levels compared to the commercial cultivar Desiree was alanine (Fig. 6). This amino acid is needed for the biosynthesis of vitamin B5 (Chakauya et al. 2006) and the formation of other metabolites essential for plant development. Moreover, it has been proposed that β-alanine is associated with the phosphorylation of starch, as well as a number of other metabolites in tubers (Carreno-Quintero et al. 2012). The content of phosphate groups in starch is related with the activity of enzymes involved in starch degradation (Blennow et al. 2002; Meekins et al. 2016) and also with the physico-chemical properties and the end-uses of starches (Blennow et al. 2002). In summary, the characterization of the metabolite composition of native accessions of potatoes from Chiloé revealed that these genotypes might be useful for understanding of metabolic processes associated with phenotypic features of interest.

4.4 Final considerations

The results presented in this study highlight the high nutritional value of the skin from these native potatoes and also indicate that the different accessions described here are promising genotypes for plant metabolic engineering and


Skin
Pulp
Amino acid
Alanine
Arginine
Asparagine
Aspartic acid
Butyric acid, 4-amino
Glutamine
Glycine
Histidine
Isoleucine
Lysine
Ornithine
Phenylalanine
Proline
Serine
Threonine
Tryptophan
Tyrosine
Valine
Organic acid
Citric acid
Fumaric acid
Glutaric acid
2-oxoglutaric acid
Glyceric acid
Isocitric acid
Itaconic acid
Maleic acid
Malic acid
Nicotinic acid
Nonanoic acid
Pyruvic acid
Succinic acid
Sugar
Cellobiose
Gentiobiose
Glucoheptose
Isomaltose
Maltose
Maltotriose
Melezitose
Melibiose
Palatinose
Raffinose
Sorbose
Turanose
Xylose
Fructose-6-P
Glucose-6-P
Ribose-5-P
Desiree
Meca de gato morada larga
Michuñe azul
Cauchau
Guicoña
Michuñe negro
Guadacho colorado
Murta
Clavela morada
Yagana
Tonta
Guadacho blanco
Lengua de vaca
Fold Change
0.0 - 0.09
0.1 - 0.19
0.2 - 0.39
0.4 - 0.59
0.6 - 0.79
0.8 -0.99
1
1 - 1.19
1.2 - 1.39
1.4 - 1.59
1.6 - 1.79
1.8 - 1.99
2.0 - 4.0
< 4.0

◂**Fig. 6** Heat map representing the changes in relative levels of primary metabolites in tissues of tubers from 11 *Solanum tuberosum* ssp. *tuberosum* L. native potato and two commercial cultivars (Desireé and Yagana). Data are scaled to the mean response measured in the Desireé cultivar for skin and pulp of each metabolite. Values shown are means of four biological replicates. Undetected metabolites are marked in gray. Different letters in each row refer to statistical differences between each genotype according to the Tukey test ($P < 0.05$)

breeding programs for enhancing beneficial health compounds in potato tubers. Furthermore, we can conclude that there is a very complex relationship between flavonoids, phenolic contents and antioxidant capacity in skin and in pulp tissues, suggesting the participation of other compounds in the antioxidant potential of potato tubers. Moreover, since site and year of cultivation affect the levels of phenolics and flavonoids, as well as antioxidant activity in potato tubers (Payyavula et al. 2012; Valcarcel et al. 2015), further experiments are essential to fully explore the results obtained in the present study. In addition, further profiling of individual phenolic and flavonoid compounds is necessary to elucidate the associations between metabolites and the genetic and mechanistic bases for them. Finally, analyses of a larger potato germplasm collection, containing more of the globe's genetic diversity, is required to validate the described correlations.

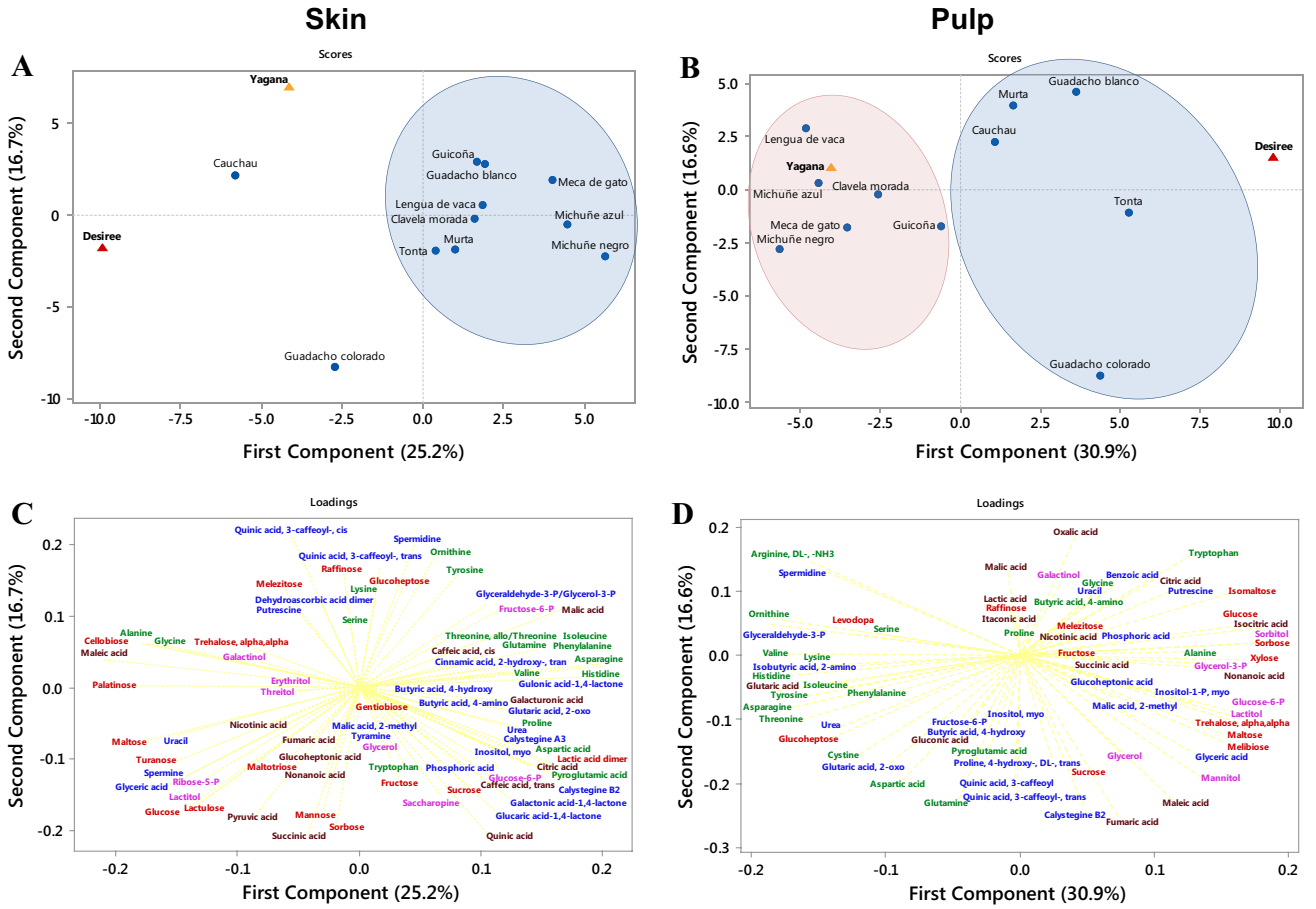


Fig. 7 Principal component analysis (PCA) in skin (**a, b**) and pulp (**c, d**) tissues of tubers from 11 native potato (*Solanum tuberosum* ssp. *tuberosum* L.) accessions and two commercial cultivars (Desireé and Yagana) based on metabolite profiles. The analysis was performed on the correlation matrix of least square means of averaged accessions. Numbers in parentheses give the percent variation explained by the first and the second principal component. **a, b** show the scores, and **c, d** show the loading plots obtained for the resulting distribution of potato genotype and metabolite data, respectively. Text colors indicate the cluster to which metabolites have been assigned using hierarchical clustering (green, amino acid; brown, organic acid; red, sugar; pink, sugar alcohol; blue, miscellaneous)

Acknowledgements This work was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) [grant number CBB - AUC-00018-16]. Research fellowships were granted by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) to ANN and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) [Grant Number CBB-BPD- 00019-16] to FMOS. ANN, MRD and CIB also acknowledge the support from the Chilean Ministerio de Educación (MEC-CONICYT; Grant PAI80160036). The authors wish to thank the NUBIOMOL-UFV for providing the facilities for the analysis of this work, and Michael Handford (Universidad de Chile) for language support.

Author Contributions CIB, MRD, and ANN conceived and designed the research. FD and CIB performed the experiments. TO, MM and FMdeOS contributed powerful analytical tools. CIB, FMdeOS and MM analyzed data. CIB and ANN wrote the manuscript. MRD, TO, JS and ARF revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animal subjects.

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Affiliations

Claudio Inostroza-Blancheteau[1,2] · Franklin Magnum de Oliveira Silva[3] · Fabiola Durán[2] · Jaime Solano[2] · Toshihiro Obata[4] · Mariana Machado[3] · Alisdair R. Fernie[4] · Marjorie Reyes-Díaz[5,6] · Adriano Nunes-Nesi[3]

[1] Núcleo de Investigación en Producción Alimentaría (NIPA-UCT), Universidad Católica de Temuco, P.O. Box 56-D, Temuco, Chile

[2] Departamento de Ciencias Agropecuarias y Acuícolas, Facultad de Recursos Naturales, Universidad Católica de Temuco, P.O. Box 56-D, Temuco, Chile

[3] Departamento de Biologia Vegetal, Universidade Federal de Viçosa, 36570-900 Viçosa-Minas Gerais, Brazil

[4] Central Metabolism Group, Max Planck Institute of Molecular Plant Physiology, Am Muehlenberg 1, 14476 Potsdam-Golm, Germany

[5] Center of Plant, Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus (BIOREN), Universidad de La Frontera, P.O. Box 54-D, Temuco, Chile

[6] Departamento de Ciencias Químicas y Recursos Naturales, Universidad de La Frontera, P.O. Box 54-D, Temuco, Chile