Electronic Journal of Biotechnology ISSN: 0717-3458
http://www.ejbiotechnology.info
DOI: 10.2225/vol16-issue6-fulltext-4 *RESEARCH ARTICLE*

Profiling carbohydrate composition, biohydrogen capacity, and disease resistance in potato

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Received June 14, 2013 / Accepted August 23, 2013
Published online: November 15, 2013

Abstract

Background: Potato (*Solanum tuberosum*) is one of the most important sources of carbohydrates in human diet. Because of its high carbohydrate levels it recently has also received attention in biohydrogen production. To exploit the natural variation of potato with respect to resistance to major diseases, carbohydrate levels and composition, and capacity for biohydrogen production we analyzed tubers of native, improved, and genetically modified potatoes, and two other tuberous species for their glucose, fructose, sucrose, and starch content.
Results: High-starch potato varieties were evaluated for their potential for *Caldicellulosiruptor saccharolyticus*-mediated biohydrogen production with Desirée and Rosita varieties delivering the highest biohydrogen amounts. Native line Vega1 and improved line Yagana were both immune to two isolates (A291, A287) of *Phytophthora infestans*.
Conclusions: Our data demonstrate that native potato varieties might have great potential for further improving the multifaceted use of potato in worldwide food and biohydrogen production.

Keywords: biohydrogen; *Caldicellulosiruptor saccharolyticus*; carbohydrates; natural variation; *Phytophthora infestans;* potato.

INTRODUCTION

There are some 190 species of wild potatoes (genus *Solanum*) in the Andes. Only a single one, *Solanum tuberosum*, has been domesticated and spread all over the world to feed it's growing population (Reader, 2008). Today, *S. tuberosum* is the fourth most important source of carbohydrates in human diet (Knapp, 2008).

In contrast to its importance in food production, potato's role as a sustainable energy source and renewable feedstock is less well known. Potato starch is an important source for paper, plastic, and glue production (Mayer and Hillebrandt, 1997; González-Gutiérrez et al. 2010). Over the past few years, the biotechnological impact of potato even further increased because of its potential value as a substrate for biohydrogen ($BioH_2$) production (Claassen et al. 1999; Mars et al. 2010). It was argued that novel, high-carbohydrate potato varieties would even be able to compete with corn, sugar beet, and other established energy plants in terms of $BioH_2$ capacity, especially when grown on marginal

soils and carrying resistance traits to the major potato diseases (Wang and Chang, 2008; Ferreira et al. 2011).

In fact, a major drawback in today's potato production is the high dependence on chemical treatments turning potato into one of the most expensive crop plants in terms of pest and disease control (Gudmestad et al. 2007). Out of the various pests and pathogens that threaten potato, late blight is still the major problem. The disease is caused by the oomycete pathogen *Phytophthora infestans*, which was the major culprit in the 1840s European, the 1845 Irish and the 1846 Highland potato famines (Nowicki et al. 2012).

To accomplish a cost-effective and good yield of potato for food production and biotechnological purposes, potato varieties with high carbohydrate levels and/or enhanced disease resistance are needed. Therefore, optimized potato varieties with desired traits are to be developed by classical breeding or by introducing useful genes through genetic transformation into an existing accepted line. Recent attempts to genetically engineer late blight resistance with genes from wild species have been effective (Song et al. 2003; Van Der Vossen et al. 2005), but it must be anticipated that the mold will soon evolve ways to overcome resistance (Jones and Dangl, 2006). Thus, to warrant continuous supply of high-carbohydrate and late blight resistance traits, permanent profiling of wild and improved varieties, and identification of genes and traits conferring these attributes, is needed.

Chile offers a huge variety of potato species and varieties with a great potential for plants with enhanced carbohydrate levels, disease resistance, and other biotech-desired traits (Solano Solis et al. 2007). Part of Chile's native potato germplasm can be obtained at local markets. In addition, Chile's Instituto de Investigaciones Agropecuarias (INIA) holds a large collection of improved potato varieties (Santos, 1990). Here, we profiled tubers of randomly selected, native and improved potato lines from Chile and the Netherlands, as well as the genetically engineered potato line AATP1 (in Desirée genetic background) with reduced activity of the plastidic ATP/ADP transporter (Tjaden et al. 1998; Linke et al. 2002; Conrath et al. 2003) for their content of glucose, fructose, sucrose, and starch. Topinambur (*Helianthus tuberosus*) with known high inulin and sucrose levels (Cheng et al. 2008; Rebora, 2008) and ulluco (*Ullucus tuberosus*) (Svenson et al. 2008) with high fructose content were also included in the assays. We evaluated the potential of the selected potato varieties Yagana, Patagonia, Rosita, Desirée, AATP1, and of Topinambur for *Caldicellulosiruptor saccharolyticus*-mediated $BioH_2$ production. *C. saccharolyticus* is a Gram-positive anaerobic bacterium that ferments a broad spectrum of mono-, di- and polysaccharides to mainly acetate, CO_2, and H_2 (Rainey et al. 1994; Bielen et al. 2013). The bacterium is known as an excellent candidate for biological H_2 production. We also compared the degree of resistance amongst the selected potato varieties to four major potato pathogens.

MATERIALS AND METHODS

Plant materials

Tubers were placed in 20L pots with standard soil (type ED 73 + Bims, Balster Einheitserdewerk GmbH, Fröndenberg, Germany). Emerging plants were grown with constant watering in a greenhouse at 20-30ºC. Tubers from 10-week-old plants were harvested and used for the assays.

Quantification of soluble sugars

Transversal sections (1 cm diameter) were punched off tubers using a corkborer. The periderm was cut off and the remaining tissue cylinder sub-divided into three equally-sized parts (left, center, right). Using mortar and pestle each part was ground to a fine powder in liquid nitrogen. Three technical replicates of 100 mg potato powder were suspended in 1 mL of distilled water and incubated for five min at 95ºC. Chilled homogenate was subjected to centrifugation in a microfuge for three min at maximum speed (12,000 g). The resultant supernatant was recovered and analyzed for the content of glucose, fructose, and sucrose by ion chromatography.

Quantification of starch

Three technical replicates each of 100 mg of ground potato powder (see above) were resuspended in 1 mL of 80% (v/v) ethanol and incubated for 60 min at 80ºC. The supernatant was evaporated by vacuum centrifugation for two hrs. The pellet was washed three times with deionized water to get rid of soluble sugars. Then, the pellet was resuspended in 100 µL deionized water. Subsequently 400 µL sodium acetate (50 mM, pH 4.7) were added. Samples were cooled down to room temperature and digested for 2 hrs at 37ºC with 5 units of each, alpha-amylase and amyloglucosidase. Digestion was stopped by heating the samples to 95ºC for 5 min. The homogenate was spun down in a microfuge for 3 min at maximum speed (12,000 g) and the supernatant collected for ion chromatography. The remaining pellet was resuspended in sodium acetate (50 mM, pH 4.7), autoclaved, and digested again with 5 units of each alpha-amylase and amyloglucosidase overnight at 37ºC to quantitatively digest starch. Digestion was stopped by incubating the samples for 5 min at 95ºC. The homogenate was spun down in a microfuge for 3 min at maximum speed (12,000 g) and the supernatant collected for the determination of starch-derived glucose by ion chromatography.

Ion chromatography

Soluble sugars (including starch-derived glucose) were quantified in an advanced Bioscan 871 liquid chromatography system (Metrohm, Filderstadt, Germany) with pulsed amperometric detection on a gold electrode and standard settings. Separation was done on a Carbo-Pac 1 column (Metrohm, Filderstadt, Germany) that was run isocratically with 0.1 M sodium hydroxide.

Processing tissue for $BioH_2$ production

For non-thermal production of $BioH_2$ from potato tubers, 50 g tuber tissue (without periderm) were homogenized in 200 mL sodium acetate (50 mM, pH 4.7) using a blender at maximum speed. The homogenate was autoclaved twice for 20 min at 1 atmosphere and at 121ºC. After samples were cooled down to room temperature, they were treated for 2 hrs at 37ºC with 200 units of each alpha-amylase and amyloglucosidase. Digestion was stopped by incubating the samples for 5 min at 95ºC. Then, the homogenate was filtered through Whatman paper using vacuum pump and funnel. The remainder was digested once again with the same activities of alpha-amylase or amyloglucosidase at 37ºC overnight and processed as described above. Filtrates were combined (total volume ~400 mL) and used for fermentation.

$BioH_2$ production by *C. saccharolyticus*

Combined filtrates were adjusted to pH 7.0 with 1 M NaOH and centrifuged to remove any remaining particles. The concentration of sugars (glucose, fructose, sucrose) and organic acids (acetate, lactate, butyrate, formic acid, propionic acid) in the combined filtrate were determined by high-performance liquid chromatography (HPLC). Total sugar concentration was determined and given as hexose equivalents. Until fermentation, the filtrate was stored at -20ºC.

Fermentation was done with the anaerobic, extreme thermophile bacterium *C. saccharolyticus* (DSM# 8903). The bacterium was grown in a medium described by Mars et al. (2010) that contained 0.9 g $[L]^{-1}$ NH4Cl2; 0.3 g $[L]^{-1}$ KH2PO4; 0.3 g $[L]^{-1}$ K2HPO4; 1 g $[L]^{-1}$ yeast extract (Serva, Germany); 2.5 mg $[L]^{-1}$ FeCl3 x 6H2O; 0.4 g $[L]^{-1}$ MgCl2 x 6H2O; 0.5 mg $[L]^{-1}$ resazurine; 0.75 g $[L]^{-1}$cysteine-HCl; 50 mM 3-(N-morpholino) propane sulfonic acid at pH 7.0, and 1 mL $[L]^{-1}$ SL-10 trace element solution (http://www.dsmz.de/). Total sugar concentration in the culture was adjusted to 6.5 g $[L]^{-1}$.

C. saccharolyticus was grown in serum flasks with a total volume of 120 mL bacterial culture. Fermentation was done in a 20 mL culture volume for 40 hrs at 72ºC. Before inoculation with 1 mL of a pre-culture the medium was flushed with N_2 to create anaerobic conditions. Pre-cultures were grown on defined medium as described above and inoculated with microorganisms from glycerol stocks which were kept at -20ºC. 1 mL-samples of the fermentation broth were taken directly after addition of the bacterial pre-culture and at 0, 16, and 40 hrs after fermentation. The fermentation was stopped by cooling the fermentation medium to room temperature. Sugar and organic acids were measured by HPLC in an Agilent 1200 system using an organic acid resin column (250 x 8 mm with organic acid

resin pre-column 40 x 8 mm, Chromatography Service, Germany) at 30ºC with 5 mM H_2SO_4 as the carrier liquid (0.5 mL $[min]^{-1}$). The volume of produced gas was measured with a gas-tight glass syringe after 16 hrs and 40 hrs. After 16 hrs gas was measured and re-injected to the flasks.

The concentrations of $BioH_2$ and CO_2 and hydrogen in the flasks head space was measured after 40 hrs by gas chromatography (GC) (Hewlett Packard 5890 series II) using a GSQ 30 m x 0.53 mm column and a GS Mol 30 m x 0.53 mm column (both Agilent J&W), respectively, with Ar as the carrier gas. The temperature of the thermal conductivity detector, injector, and columns was kept at 210ºC, 150ºC, and 70ºC, respectively.

Pathogen susceptibility assays

Two isolates of *P. infestans* from Chilean fields (A287 and A291; mating type A1), R. *solani* AG3, and *F. solani* were used to assay the disease susceptibility of different potato varieties. For the assays plants were grown at 22ºC in a 16 hrs light period. Fully expanded, detached leaves were placed in a 12 x 12 cm plastic plate containing sterile agar medium (15 g $[L]^{-1}$ with 0.1 µg $[L]^{-1}$ kinetin). Leaves were inoculated with 40 µL of a *P. infestans* spore suspension (50,000 spores $[mL]^{-1}$). Seven days later, the size of developing lesions was measured upon trypan blue staining as described by Vogel and Somerville (2000). Ten randomly selected lesions were evaluated microscopically with a 20x objective. Lesion area was quantified with the area selection tool of the Diskus software. Lesion area was scaled from very low (scale 1) to very high (scale 5).

To assay the interaction of potato varieties with *R. solani* an agar piece (5 x 5 mm) with cultivated *R. solani sclerotia* was put onto detached leaves (Gutiérrez et al. 2009). The size of developing lesions and a halo was measured with a ruler at 72 hrs post inoculation.

To assay the interaction of potato leaves with *F. solani*, leaves were inoculated by placing 20 µL spore suspension (25,000 spores $[mL]^{-1}$) on the leaf surface. The size of developing lesions and a decolorized halo were measured with a ruler also at the 72 hrs-time point post inoculation.

The interaction of potato with *P. carotovorum* was evaluated in a tuber assay with potato discs. Tuber discs (1 cm diameter, 0.5 cm thickness) were punched out from intact tubers and inoculated as described by Linke et al. (2002). Weight loss of the discs was determined 72 hrs post inoculation. Susceptibility estimation was based on visual inspection and assigned to one of five categories ranging from very low (scale 1) to very high (scale 5) susceptibility.

RESULTS

Collection of plants

We obtained potato tubers with different phenotypes in terms of shape and color (Figure 1) from a local market (Vega Central) in Santiago, Chile. Based on where they were bought or where they came from, they were given common names: “Vega1”, “Vega2”, “Carahue”, “Gigante”, “Temuco”, and “Rosita”. In addition, improved lines “R89054-34”, “Yagana” and “Patagonia” were obtained from INIA. Improved commercial varieties “Bintje” and “Desirée” were purchased from Gärtner Pötschke internet store (www.poetschke.de). Tubers of Topinambur and ulluco (Figure 1) were bought at Santiago Vega Central market. Transgenic AATP1 line (in Desirée genetic background) was engineered in the Neuhaus laboratory (Kaiserslautern Tech University, Germany).

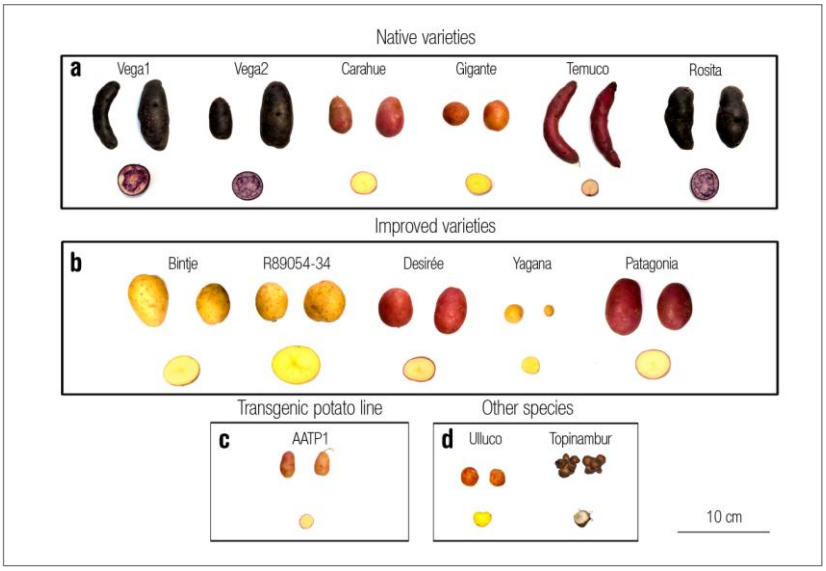

Fig. 1 Phenotype of tubers from species and potato varieties used in this study. (a) Native potato lines were bought at Vega Central Market in Santiago, Chile. (b) Improved potato varieties were from INIA or an internet store (http://www.poetschke.de/). (c) Transgenic AATP1 line was engineered in the Neuhaus laboratory. (d) Topinambur and ulluco were from Vega Central Market.

Different carbohydrate levels in diverse tuberous plants

Tuber tissue of the above mentioned species and potato varieties were homogenized and tested for their content of glucose, fructose, and sucrose by ion chromatography (Figure 2a).

The profiles of the three soluble sugars revealed that, when compared to improved varieties, tubers of the native potato varieties Vega1, Vega2, Carahue, Gigante, Temuco, and Rosita all have low levels of any of the three sugars assayed (Figure 2a). Among the selected native varieties, the highest amount of free glucose (~4 mg [g fresh weight]$^{-1}$) was found in Gigante, while Carahue and Vega2 displayed lower glucose amounts. Gigante and Vega2 expressed the highest sucrose levels among the selected wild varieties (Figure 2a). Fructose was hardly detected in any of the native tubers, except in Carahue with slightly enhanced fructose level (Figure 2a).

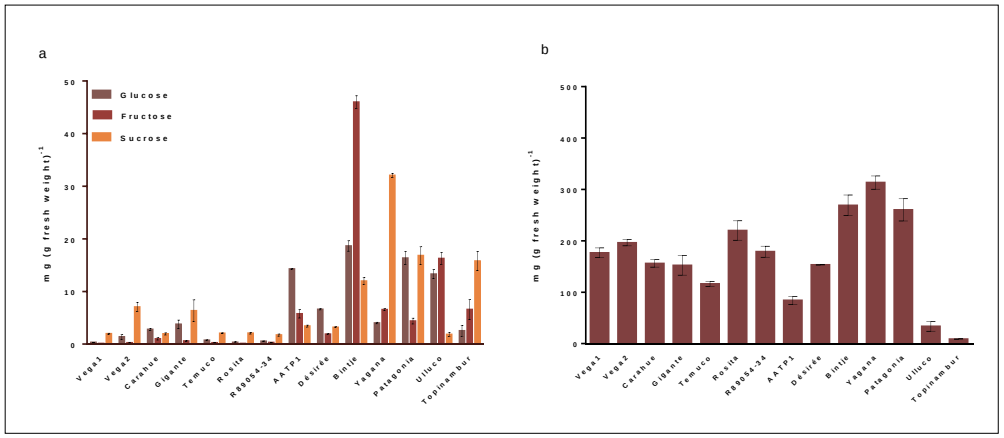

Fig. 2 Carbohydrate levels in diverse tuberous plants. (a) Levels of glucose, fructose, and sucrose in tuber tissue of the given tuberous species and potato varieties. (b) Amount of glucose released upon serial digestion of starch from tuber tissue with alpha-amylase and amyloglucosidase.

Among the improved potato varieties, Bintje tubers had a very high level of fructose (> 45 mg [g fresh weight]$^{-1}$) (Figure 2a). The fructose content was lower in ulluco (~16 mg [g fresh weight]$^{-1}$) and even lower in Yagana, AATP1, and Topinambur. Variety Desirée displayed the lowest fructose level among the improved varieties, but Desirée's fructose content was still higher than in any of the native potato varieties (Figure 2a).

The highest sucrose content was found in the improved Yagana variety. Sucrose levels were still high in Patagonia, Bintje, and Topinambur. Only little sucrose was found in AATP1, Desirée, and ulluco with levels lower than in the varieties lines Vega2 and Gigante (Figure 2a).

Glucose levels were high (15-20 mg [g fresh weight]$^{-1}$) in Bintje, Patagonia, AATP1, and ulluco, while Desirée, Yagana, and Topinambur displayed lower glucose levels. However, the level of glucose in these plants was in the same range, or even higher than in Gigante and Carahue which both display the highest glucose levels among the native potato varieties analyzed (Figure 2a).

The content of starch in tubers of the investigated plants was assayed as the amount of glucose released upon serial digestion with alpha-amylase and amyloglucosidase (Batey, 1982). As shown in Figure 2b, the highest starch content was found in tubers of the Yagana variety. Bintje, Patagonia, and Rosita also showed high starch levels, which were intermediate in Vega2, R89054-34, Vega1, Carahue, Gigante, and AATP1. Tubers of ulluco and Topinambur displayed low or very low starch levels.

Caldicellulosiruptor saccharolyticus-mediated $BioH_2$ production

Because of their different content in monomeric sugars (Figure 2a) and starch (Figure 2b), tuber homogenates of selected improved potato varieties Desirée, Yagana, Patagonia, transgenic line AATP1, native potato variety Rosita, and of Topinambur were used for *C. saccharolyticus*-mediated $BioH_2$ production. *C. saccharolyticus* is a strictly anaerobic, thermophylic bacterium that hydrolyzes a variety of carbohydrates to produce $BioH_2$, organic acids, and CO_2.

Desirée tuber tissue yielded 1.084 mmol BioH2 after 40 hrs of fermentation (Figure 3a) corresponding to 1.93-fold the yield of a positive control in which a defined aqueous glucose solution (6.5 g [L]$^{-1}$ glucose) was used for fermentation. Tuber tissue of the Rosita native variety was number two amongst the varieties tested (Figure 3a), yielding 0.662 mmol $BioH_2$ corresponding to 1.18-fold the yield of the glucose control. Tuber tissue of potato varieties Yagana and Patagonia, transgenic line AATP1, and of Topinambur provided lower $BioH_2$ amounts. They were similar to those of the positive glucose control (Figure 3a).

The varying capacity for $BioH_2$ production between different tuberous species and varieties was not reflected by their total sugar consumption, which was high in the glucose control and Desirée variety, intermediate in Topinambur, Rosita, and AATP1 and low in Yagana and Patagonia (Figure 4). The production of acetate was highest when tuber tissue of the Desirée, Rosita, Yagana and Patagonia varieties were used for fermentation with levels in the 0.8 mmol acetate [mmol organic acid]$^{-1}$ range (Figure 3c). Acetate accumulated to lower levels when tuber filtrates of the AATP1 potato line or of Topinambur were fermented (Figure 3c). However, tubers of these two plants gave rise to the highest levels of lactate upon fermentation (Figure 3b). Lactate levels were only low upon filtrate's fermentation from the Desirée, Rosita, Yagana, and Patagonia varieties (Figure 3b).

Interaction of *S. tuberosum* varieties with major potato pathogens

To evaluate the interaction of native and improved potato varieties as well as the transgenic AATP1 potato line with four major potato pathogens, their response to inoculation with two Chilean isolates (A291, A287) of *P. infestans*, *Rhizoctonia solani* and *Fusarium solani* was tested. The hemibiotrophic oomycete *P. infestans* causes potato blight and the fungus *R. solani* elicits brown canker on potato shoots and leaves (Osusky et al. 2005; Rivero et al. 2012). *F. solani* is another economically important fungal pathogen causing yellowish mottling and bronzing on potato leaves (Helgeson et al. 1998).

In addition to the interaction of these pathogens with leaves, we evaluated the response of tubers of the different potato varieties to the bacterium *P. carotovorum*, the causal agent of soft-rot disease on potato tubers (Agrios, 2005). Evaluation of disease ratings was based on visual inspection and assigned to one of five categories ranging from very low (scale 1) to very high (scale 5) susceptibility to disease.

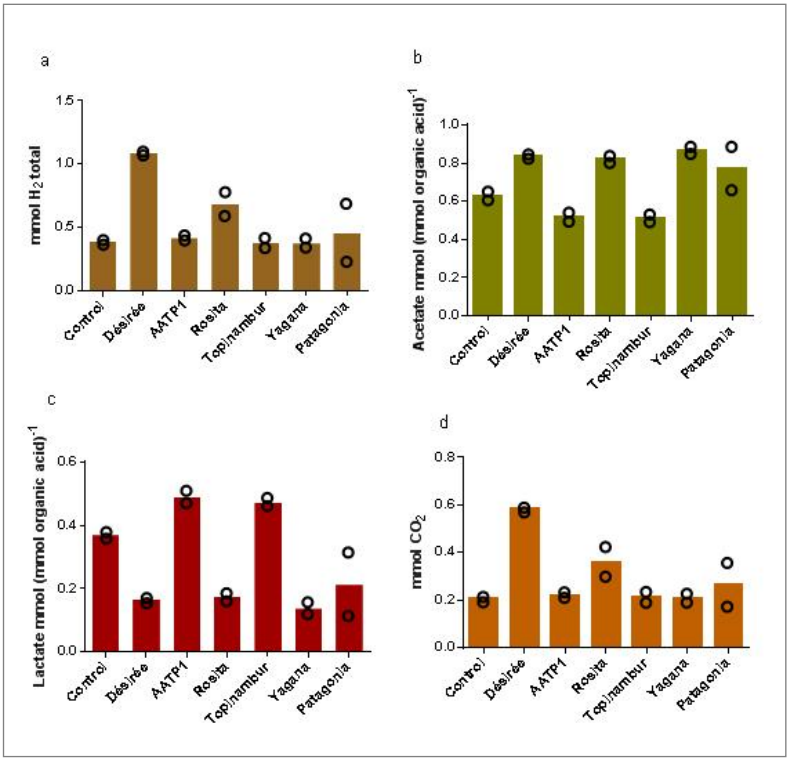

Fig. 3 Production of gases and organic acids by *C. saccharolyticus*. (a) Production of BioH_2upon fermentation of tuber tissue from given species and varieties by *C. saccharolyticus*. (b) Production of lactate. (c) Production of acetate. (d) Production of CO_2.

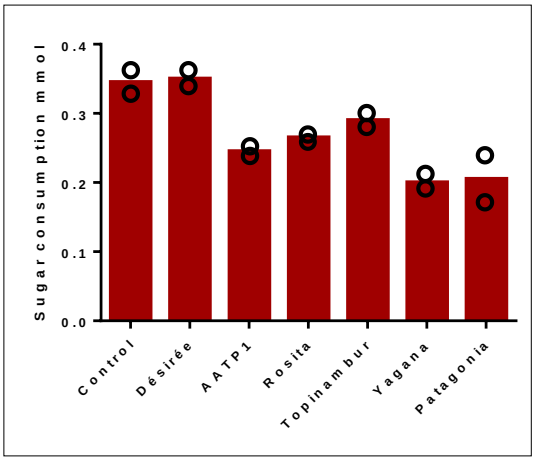

Fig. 4 Total sugar consumption in Topinambur and selected potato varieties. Average values of two independent measurements are shown (circles).

As is shown in Table 1, improved potato variety Yagana showed the lowest average susceptibility index to disease with very good resistance to both isolates of *P. infestans* and *R. solani*, intermediate resistance against *F. solani*, and little resistance to soft rot disease. Amongst the varieties tested, native variety Gigante was number two in terms of average disease resistance with very good resistance against *R. solani* and *F. solani* and medium resistance to the two *P. infestans* isolates (Table 1). Vega1 and R89054-34 also showed good average resistance to potato pathogens with R89054-34 performing very well against *P. carotovorum* and Vega1 showing very good or good resistance to the two *P. infestans* isolates. Most other varieties had intermediate resistance to the major spud diseases, except Temuco, Rosita, and especially Vega2 which was highly susceptible to all pathogens tested (Table 1). It is noteworthy that Desirée showed medium resistance to *P. carotovorum* and high or very high susceptibility to the other pathogens tested (including the two *P. infestans* isolates) while transgenic potato line AATP1 had good-to-medium resistance to the two P*. infestans* strains and to *F. solani* yet displayed very low-to-low resistance to *P. carotovorum* and *R. solani* (Table 1).

Table 1. Susceptibility assays of native and improved lines to major potato pathogens. Susceptibility 1: Very low; 2: Low; 3: Medium; 4: High; 5: Very high.

		***P. infestans* A291**	***P. infestans* A287**	***R. solani***	***P. carotovorum***	***F. solani***	**Average susceptibility**
Native varieties	**Vega 1**	2.0	1.0	3.0	3.0	5.0	2.8
	Vega 2	5.0	4.0	5.0	5.0	4.0	4.6
	Carahue	4.0	5.0	3.0	3.0	5.0	4.0
	Gigante	3.0	3.0	1.0	5.0	1.0	2.6
	Temuco	5.0	5.0	4.0	4.0	4.0	4.4
	Rosita	5.0	5.0	4.0	3.0	5.0	4.4
Improved varieties	**R89054-34**	3.0	3.0	4.0	1.0	3.0	2.8
	Aatp1	2.0	3.0	5.0	4.0	3.0	3.4
	Desirée	4.0	5.0	4.0	3.0	5.0	4.2
	Bintje	4.0	5.0	3.0	4.0	4.0	4.0
	Yagana	1.0	1.0	1.0	5.0	3.0	2.2
	Patagonia	3.0	3.0	4.0	2.0	4.0	3.2

DISCUSSION

The improved potato varieties Bintje, Yagana, and Patagonia had the highest levels of both monomeric sugars and starch amongst all species and potato varieties tested (Figure 2). In Yagana, the enhanced carbohydrate level was associated with high average resistance to the major potato diseases (Table 1). These findings are consistent with the “high sugar resistance” concept claiming a causal relationship of enhanced levels of soluble carbohydrates with plant disease resistance (Horsfall and Dimond, 1957). However, since the potato varieties Bintje and Patagonia, which both also have enhanced levels of soluble sugars and starch (Figure 2), express only medium or low resistance to the major spud diseases (Table 1), the validity of the “high sugar resistance” concept is arguable. The concept was also questioned by Herbers et al. (1996) who demonstrated that tobacco leaves ectopically expressing cytosolic yeast invertase had enhanced glucose levels yet they missed enhanced resistance to potato virus Y. Since other transgenic tobacco lines that localized yeast invertase to the cell wall or vacuole also expressed high glucose levels but had enhanced potato virus Y resistance, the authors concluded that the localization of soluble carbohydrates within the cell’s endomembrane system, rather than the accumulation of soluble sugars per se, is required for enhanced plant defense (Herbers et al. 1996).

The finding that transgenic potato line AATP1, which is engineered in the Desirée genetic background, expressed lower average disease susceptibility than Desirée with particularly higher resistance to late blight disease is consistent with our earlier findings (Linke et al. 2002; Conrath et al. 2003). However, the enhanced susceptibility to *P. carotovorum* seen in AATP1 contrasts our earlier results. The reason for the difference in results is currently unclear.

Because Vega1 expressed high resistance to potato blight and since Gigante effectively warded off *R. solani* and *F. solani* (Table 1), these two native varieties are potential new sources for the development, by classical breeding or transgenic approaches, of optimized *S. tuberosum* varieties for cost-effective and sustainable potato production. A recent gene technological attempt to confer late blight resistance to an existing, accepted potato line by introducing blight resistance genes from the Mexican wild potato *Solanum bulbocastanum* has been effective (Song et al. 2003; Van Der Vossen et al. 2005). However, it must be anticipated that the late blight will soon evolve ways to overcome the resistance (Jones and Dangl, 2006) and therefore, identification of further blight resistance genes, such as those from the Yagana and Vega1 varieties, is needed.

Of all species and varieties tested, potato varieties Rosita and Desirée yielded the highest amounts of *C. saccharolyticus*-produced $BioH_2$. This was kind of a surprising result since Rosita and Desirée were low in soluble sugars and had only moderate levels of starch (Figure 2) which both are major sources for *C. saccharolyticus*-mediated $BioH_2$ release (Rainey et al. 1994). We do not know why the levels of soluble carbohydrates and/or starch do not correlate with the potential of tubers for $BioH_2$ yield. It might be speculated that carbohydrates other than glucose, fructose, and sucrose and/or carbohydrate polymers other than starch may be present in high amounts in Rosita and Desirée and serve as a substrate for *C. saccharolyticus*-produced $BioH_2$ production.

The metabolism of *C. saccharolyticus* shifts from acetate to lactate biosynthesis if fermentation conditions are suboptimal, *e.g.* when bacterial growth is inhibited by accumulating $BioH_2$ (Van Niel et al. 2003; Willquist et al. 2010) or when growth reaches the stationary state (Van Niel et al. 2003). Therefore, acetate and lactate levels may indicate this metabolism shift. Hence, relatively high acetate fractions indicate that the culture did not shift its metabolism yet, or has shifted only a short time before measurement. According to this, total organic acids production can be lower between two runs with the same carbon source while the acetate production remains the same as is the case for Rosita.

In sum, our data demonstrate that wild and improved potato varieties are valuable sources for the identification of genes and traits that might be useful in targeted approaches to provide novel potato varieties with augmented monomeric and/or polymeric carbohydrate levels, enhanced disease resistance, and increased potential for $BioH_2$ production.

ACKNOWLEDGMENTS

We would like to thank Katharina Göllner and Dennis Hopkins for doing pilot experiments. We acknowledge the Instituto de Investigaciones Agropecuarias from Chile (INIA) for providing potato varieties and we are thankful to Ulrike Steiner for providing fungal pathogens.

Financial support: This work was supported by a joint Comisión Nacional de Investigación Científica y Tecnológica (CONICYT) / Deutsche Forschungsgemeinschaft (DFG) 062-2008 program, and CONICYT Fellowship D-2108007 to SDM. SDM is a member of the Biotechnology Ph.D. Program at Universidad Andres Bello.

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How to reference this article:

DIEZ-DE-MEDINA ROLDÁN, S.; SILVA, H.; JEBLICK, W.; NOWIK, I.; MODIGELL, M.; NEUHAUS, H.E. and CONRATH, U. (2013). Profiling carbohydrate composition, biohydrogen capacity, and disease resistance in potato. *Electronic Journal of Biotechnology*, vol. 16, no. 6. http://dx.doi.org/10.2225/vol16-issue6-fulltext-4