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Impact of light-exposure on the metabolite balance of transgenic potato tubers with modified glycoalkaloid biosynthesis

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ABSTRACT

Metabolite profiling (liquid chromatography–mass spectrometry (LC–MS) and gas chromatography (GC–MS)) was used to assess the impact of light on the composition of transgenic potato (*Solanum tuberosum* L. *cv.* Desirée) with reduced glycoalkaloid content *via* the down-regulation of the *SGT*1 gene. Transgenic tubers exhibited an almost complete knock-out of α-solanine production and light had little impact on its accumulation. Levels of α-chaconine increased significantly in the peel of both the control and transgenic lines when exposed to light, particularly in the transgenic line. Major differences in metabolite profiles existed between outer and inner tuber tissues, and between light and dark-treated tubers. Many of the light-induced changes are explicable in terms of pathways known to be affected by stress responses. The impact of transgenesis on profiles was much less than that of tissue type or light and most differences were explicable in terms of the modification to the glycoalkaloid pathway.

1. Introduction

Steroidal glycoalkaloids are naturally occurring toxins produced by plants of the *Solanaceae* family. Tubers of cultivated potato (*Solanum tuberosum* L.) accumulate the two predominant glycoalkaloids α-chaconine and α-solanine, which can constitute as much as 95% of the total glycoalkaloids present in the potato plant (van Gelder, 1990). Glycoalkaloids are proposed to contribute to insect and/or microbial pest defence but can also affect product quality (flavour) and safety if present at elevated levels. Glycoalkaloids are not evenly distributed throughout the tuber, with the highest concentrations usually associated with areas that are undergoing high metabolic activity, such as sprouts, peels and the area around the potato 'eyes'. Small immature tubers are normally high in glycoalkaloids, since they are still metabolically active. The majority of glycoalkaloids are located within the first 1 mm from the outside surface of the tuber, with concentrations decreasing towards the centre of the tuber (Friedman, 2006 and references therein). The total glycoalkaloid content of potato commonly ranges between 10 and 150 mg/kg fresh weight (van Gelder, 1990). However, a variety of environmental, genetic and stress factors, such as insect attack, disease, light, maturity, mechanical damage, and storage temperatures have been reported to lead to elevated tuber glycoalkaloid content (Sinden, Sanford, & Webb, 1984).

Potato glycoalkaloids are structurally similar compounds, which share the same aglycone, solanidine, but differ in their carbohydrate component. They contain either glucose (α-chaconine) or galactose (α-solanine) as the primary glycosyl residue. Fig. S1 gives an overview of glycoalkaloid biosynthesis in cultivated potato.

The biosynthesis of γ-solanine is catalysed by the enzyme UDP-galactose:solanidine galactosyltransferase (designated SGT1; McCue et al., 2005) from UDP-galactose and solanidine. The biosynthesis of γ-chaconine from UDP-glucose and solanidine (Bergenstråhle, Tillberg, & Jonsson, 1992; McCue et al., 2005; Stapleton, Allen, Friedman, & Belknap, 1991; Zimowski, 1991) is catalysed by the enzyme UDP-glucose:solanidine glucosyltransferase (designated SGT2; McCue et al., 2006). The biosynthesis of both α-solanine and α-chaconine is catalysed by the enzyme UDP-rhamnose:β-steroidal glycoalkaloid rhamnosyltransferase (designated SGT3; McCue et al., 2007).

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Exposure of potato tubers to light results in the conversion of amyloplasts to chloroplasts (Anstis & Northcote, 1973), increased metabolic activity and greening. Whilst chlorophyll and the other photosynthetic pigments produced are tasteless and harmless, green potatoes are generally considered unfit for consumption, due to the associated increase in the synthesis of the colourless glycoalkaloid solanine when exposed to light (Grunenfelder, Hiller, & Knowles, 2006). These levels are affected by both the intensity of light and length of exposure – the latter being an important factor in greening because of the cumulative effect. Greening is also affected by other factors, such as variety, maturity status of the potato, storage conditions and temperature. However, the extent of greening is not a direct measure of a potato's glycoalkaloid content, as it has been reported that there is no direct metabolic connection between chlorophyll and glycoalkaloid accumulation (Edwards, Saint, & Cobb, 1998). The two processes occur concurrently but are biochemically independent.

The authors have previously reported on a GM potato line '*sgt9-2*', with the *SGT*1 gene down-regulated, which exhibits an almost complete knock-out of α-solanine (McCue et al., 2005; Shepherd et al., 2015). To fully assess the efficacy and stability of this knock-out, this study examines the impact of exposing tubers of both this transgenic line and its associated control line to continuous light for 4 or 11 days. Targeted analyses focus on glycoalkaloid and chlorophyll formation, whilst complementary untargeted analyses using LC–MS and GC–MS (polar and non-polar fractions), should allow us to more fully assess the impact of light exposure in general (and on the response of the *sgt9-2* line specifically), on the tuber metabolome.

2. Materials and methods

2.1. Plant material

Explants of potato (*S. tuberosum* L.) *cv.* Desirée were obtained by axenic micro-propagation on Murashige and Skoog medium under artificial heating (22 °C) and lighting (16 h light:8 h dark cycle, 100 μmol/m^2/s photon flux density). Plants were sub-cultured regularly (every 4 weeks) by axenically transferring actively growing apices to fresh medium. The transgenic line and a control line transformed with an "empty vector" construct – vector pBINPLUS-ARS (*BIN+*) containing the *npt*II gene, but not the *SGT*1 antisense cDNA, were regenerated through tissue culture *via* a callus phase.

2.2. Construction of transgenic plants

The transgenic line used in this study was developed using the binary vector pBINPLUS-ARS (Belknap, Rockhold, & McCue, 2008). The construct contained an antisense fragment of cDNA for *SGT*1, driven by the tuber-specific granule bound starch synthase (GBSS6) promoter (van der Steege, Nieboer, Swaving, & Templaar, 1992); and the neomycin phosphotransferase II (*npt*II) marker gene. Cloning of the SGT1 cDNA and binary plasmid assembly of the constructs used here has been previously reported (McCue et al., 2005). *Agrobacterium*-mediated transformation (Gustafson et al., 2006) was used to generate putative transgenic lines, selected by growth on kanamycin. Transgenesis was confirmed by PCR (data not shown). It is well known that tissue culture can give rise to somaclonal variation (Davies, 2002), a possible source of unintended effects independent of *Agrobacterium*-mediated gene transfer. An empty vector line developed and grown alongside the GM line with targeted trait modifications represents an important control for vegetatively propagated plants where a comparison between matched homozygous and azygous plants is not possible.

In a single year, plants were grown from tubers in a glasshouse in 30-cm diameter pots containing UC (University of California) compost and watered with automatic irrigation. For both the transgenic (*sgt9-2*) and control (*BIN+14-3*) line five seed tubers were planted. Plants were harvested at natural senescence and the tubers from the five pots (per line) pooled to give one bulk sample per line. Tubers were stored at ambient temperature (*ca.* 10 °C) for two weeks in the dark to facilitate skin set.

2.3. Light exposure treatment of sgt9-2 and BIN+14-3 tubers

Four trays were lined with moist paper towelling. Eight tubers (numbered 1–8) from the transgenic line *sgt9-2* were cut in half longitudinally. One half of each of the eight tubers was placed cut surface down on one of two separate trays (numbered 1 and 2), so that two trays contained halves from all eight tubers. This process was repeated using eight tubers (numbered 9–16) of the control line *BIN+14-3* divided into two other trays (numbered 3 and 4), so that each tray contained eight tuber halves (Fig. S2).

Two trays, one containing *sgt9-2* (tray 1) and one containing *BIN+14-3* (tray 3) tuber halves, were placed in an environmental chamber set at 20 °C, with ambient relative humidity and illuminated with high-pressure sodium lights (predominant wavelengths 550–650 nm) with a photon flux density of 140 μmol m^{-2}/s at tray level. The other two trays containing *sgt9-2* (tray 2) and *BIN+14-3* (tray 4) tuber halves were placed carefully in ventilated black bin liners (to eliminate any light) and placed in the same environmental chamber as described above.

After 96 h (4 days) of light exposure four tuber halves of either the control line (*BIN+14-3*) or the *sgt9-2* line were selected and opposite pairs bulked to supply two biological replicate units of material for analysis. The same process was followed for the complementary halves maintained in darkness. For each bulked pair the outer tissues were removed by cutting along the xylem ring (*ca.* 1 cm in depth from the surface), and was designated the 'peel'. The remaining inner tissues were designated the 'flesh'. After 264 h (11 days) of light exposure the remaining tuber halves from each tray were bulked in the same way. Peel and flesh were individually diced, weighed and frozen in liquid nitrogen. Frozen tissues were freeze-dried for one week prior to milling using a mill (Tecator Udy) fitted with a 1-mm sieve. Milled freeze-dried potato powders were stored in re-sealable bags at −20 °C (in the dark) until required for analyses. Metabolomic analyses have shown that storage in this way has no impact on metabolite profiles for at least three months (Shepherd et al., 2007).

2.4. Targeted analysis: chlorophyll and glycoalkaloid content

Freeze-dried peel and flesh were analysed for total chlorophyll content as outlined by Lichtenthaler and Wellburn (1983). Chlorophylls were extracted from 1 g freeze-dried potato powder (flesh and peel) using 5 mL 85% (*v*/*v*) aqueous acetone, and the absorbance (*A*) of the resulting supernatant measured at 663 nm and 646 nm in a 1-cm cuvette placed in a spectrophotometer. Total chlorophyll (mg/L) was calculated using the formula of Harborne (1988), Chapter 5:

$$\text{Total chlorophyll(mg/L)} = (17.3 * A_{646}) + (7.18 * A_{663})$$

Glycoalkaloids were extracted from 500 mg freeze-dried potato powder (flesh and peel) using 10 mL 2% (*v*/*v*) aqueous acetic acid containing 0.5 g sodium bisulphite per 100 mL. After centrifugation, the supernatant was loaded onto a pre-equilibrated 100 mg C18 reverse-phase column, washed with 15:85 *v*/*v* acetonitrile/0.05 M phosphate buffer and the glycoalkaloid contents of