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Research article

Changes in potato phenylpropanoid metabolism during tuber development

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ABSTRACT

Phenylpropanoid metabolite and transcript expression during different developmental stages were examined in field grown potatoes. Carbohydrate and shikimic acid metabolism was assessed to determine how tuber primary metabolism influences phenylpropanoid metabolism. Phenylpropanoid concentrations were highest in immature tubers, as were some transcript levels and enzyme activities including phenylalanine ammonia lyase (PAL). Phenylpropanoid concentration differences between mature and immature tubers varied by genotype, but in some cases were approximately three-fold. The most abundant phenylpropanoid was chlorogenic acid (5CGA), which decreased during tuber maturation. Hydroxycinnamoyl-CoA:quinate hydroxycinnamoyl transferase (HQT) transcripts were highly expressed relative to other phenylpropanoid genes, but were not well correlated with 5CGA concentrations ($r = -0.16$), whereas HQT enzyme activity was. In contrast to 5CGA, less abundant chlorogenic isomers increased during development. Concentrations of hydroxycinnamic acid amides were higher in immature tubers, as was expression of arginine- and ornithine decarboxylases. Expression of several genes involved in carbohydrate or shikimate metabolism, including sucrose synthase and DAHP, showed similar developmental patterns to phenylpropanoid pools, as did shikimate dehydrogenase enzyme activity. Sucrose, glucose and fructose concentrations were highest in immature tubers. Exogenous treatment of potatoes with sugars stimulated phenylpropanoid biosynthesis, suggesting sugars contribute to the higher phenylpropanoid concentrations in immature tubers. These changes in phenylpropanoid expression suggest the nutritional value of potatoes varies during development.

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1. Introduction

Physiological roles of potato (*Solanum tuberosum* L.) phenylpropanoids include enabling plants to cope with biotic and abiotic stresses [1]. Extensive variance in phenylpropanoid content occurs among potato germplasm, reflecting the considerable genetic diversity of potatoes and the capacity of tubers to synthesize these compounds [2,3]. PAL has been well studied in tubers, and increases in response to light, infection or treatment with elicitor [4,5]. PAL activity and phenylpropanoids increase after wounding, during which a suberized wound periderm is formed [6,7]. Differential expression of phenylpropanoid genes was observed in tubers exhibiting white and purple sectoring [8].

Apart from physiological roles, phenylpropanoids influence the nutritional value of potatoes, with one study showing white potatoes to be the third largest source of dietary phenolics in the American diet [9]. Phenylpropanoids can have antioxidant capacity or other health-promoting characteristics [10–12]. Chlorogenic acid (5CGA, Fig. 1), the most abundant phenolic in potatoes, may

Abbreviations: ADC, arginine decarboxylase; AMY, alpha-amylase; BDCS, bis(-dihydrocaffeoyl)spermine; BDCSD, bis(dihydrocaffeoyl)spermidine; C3H, *p*-coumarate 3-hydroxylase; C4H, cinnamate 4-hydroxylase; CHI, chalcone isomerase; CP, caffeoyl putrescine; CGA, chlorogenic acid; DAHP, 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase; DAP, days after planting; diCGA, dicaffeoylquinic acid; FP, feruloyl putrescine; FQA, feruloylquinic acid; HCT, hydroxycinnamoyl; CoA, shikimate hydroxycinnamoyl transferase; HQT, hydroxycinnamoyl-CoA:quinate hydroxcinnamoyl transferase; INV, soluble acid invertase; Kmp, kaempferol-3-rutinose; ODC, ornithine decarboxylase; PAL, phenylalanine ammonia lyase; PGK, phosphoglycerate kinase; Phe, phenylalanine; PPO, polyphenol oxidase; Rut, rutin; SAG, salicylic acid glucoside; SP, starch phosphorylase; SUSY, sucrose synthase; SSY, soluble starch synthase; TDCS, tris(dihydrocaffeoyl)spermine; TDCSD, tris(dihydrocaffeoyl)spermidine.

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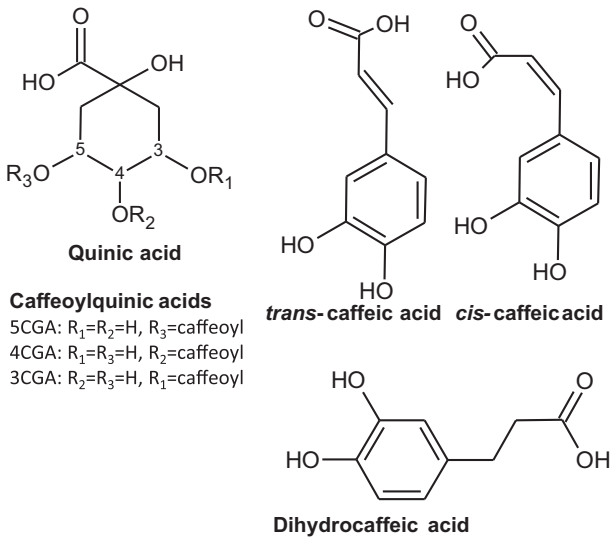

Fig. 1. Structures of hydroxycinnamic metabolites.

have various health-promoting effects, including reduced risk of some cancers, cardiovascular disease [13], hypertension [14], and diabetes [15].

Engineering the tuber phenylpropanoid pathway has the potential to further increase the nutritional value of potatoes [16]. Little is known about phenylpropanoid metabolism in the latter stages of tuber maturation, especially when grown under cropping conditions. A more comprehensive understanding of phenylpropanoid metabolism during tuber maturation in actual field conditions could provide insights about approaches to produce more nutritious potatoes. Because tubers are a staple food consumed in quantity, even minor differences in phenylpropanoid content may be dietarily significant. Hydroxycinnamic acids vary during development in cherries and decreased sharply during fruit development in apples and pears [17,18]. Tuber periderm was reported to have elevated amounts of phenylpropanoids during mid-season bulking and folate was higher in immature tubers [19,20]. Moreover, sucrose stimulates phenylpropanoid expression in plants, and there are large changes in sucrose concentrations during tuber development [21–23]. In this study, we examined whether tuber maturation over the course of the growing season modulates phenylpropanoid expression and explored potential interactions between sugar and phenylpropanoid metabolism as tubers developed from early bulking through to full maturity.

2. Results

2.1. Phenolics during tuber maturation

Phenylpropanoid metabolism was characterized throughout the course of tuber development starting when tubers were ca. 8–28 g (2–3 cm diameter) and ending with fully developed and physiologically mature tubers averaging 180–250 g. The time course of foliar and tuber development, along with tuber specific gravity (dry matter) and carbohydrate (suc, glc, fru) content were analyzed as biochemical indices of tuber maturity (Fig. 2). Collectively, the data in Fig. 2 and Supplemental Table 1 explicitly define the relative physiological maturities of 'Defender', 'Umatilla Russet', and 'Russet Burbank' tubers used for phenylpropanoid analysis at each timepoint throughout the season. Foliar growth was rapid during the first half of the growing season; reaching a maximum at 81–84 DAPS and then declining through 146 DAP for all three cultivars. Increases in tuber yields and average tuber fresh weights were linear through the bulking phase (64–131 DAP) of tuber development (Fig. 2). Tuber growth then slowed appreciably during the maturation phase (ca. 130–170 DAP) of development. Tuber physiological maturity was attained 148, 151, and 146 DAP for 'Defender', 'Umatilla Russet', and 'Russet Burbank' tubers, respectively (Fig. 2).

The concentration of total phenolics was highest at the first developmental stage (60–64 DAP) when 'Defender', 'Umatilla Russet' and 'Russet Burbank' tubers averaged 8–28 g fresh weight (Fig. 3A). Phenolic concentrations fell as tubers developed through to full maturity (stage 10, 170 DAP) in all cultivars. The decrease between stage 1 (64 DAP) and stage 10 (170 DAP) ranged from 16% in 'Defender' to 44% in 'Umatilla Russet.' Phenolic content decreased the most from 64 to 92 DAP, a period consistent with the attainment of maximum foliar development, and then stabilized during the remainder of tuber bulking. A similar trend was evident for 'Green Mountain' tubers. The higher amount of phenolics observed in 'Green Mountain' may be due to both genotypical and environmental components. Concentrations remained high in tubers grown in a different year (data not shown).

The cultivars depicted in Fig. 3A are white-flesh potatoes. To determine whether the overall trend of declining phenylpropanoid concentration with tuber maturity is broadly representative of potato germplasm, five additional genotypes with purple- or yellow-flesh color were examined. Immature potatoes harvested ~75 DAP were compared to mature tubers harvested ~160 DAP from the same location. All five genotypes showed higher concentrations of phenolics in immature tubers (Fig. 3B). The biggest differences in phenolic content between immature and mature tubers were seen in the purple-flesh cultivars, which also had the highest amounts of total phenylpropanoids. The decrease in phenolic compounds during tuber growth occurred on a fresh weight and dry weight basis, indicating that changes were not simply a consequence of starch accumulation during development.

2.2. Shikimate and PAL

To assess changes in phenylpropanoid metabolism during tuber maturation and relationships between secondary and primary metabolism, metabolite pools and expression of key genes within the phenylpropanoid pathway, shikimate or carbohydrate metabolism were measured (Fig. 4). PAL catalyzes the first committed step in the biosynthesis of most phenylpropanoids by converting phenylalanine derived from the shikimate pathway to trans-cinnamic acid and ammonia [24]. Unlike total phenolics, concentrations of phenylalanine were relatively stable during tuber development in three of the four genotypes and did not show a clear trend to decrease (Fig. 5A). PAL activity is regulated transcriptionally and post-transcriptionally [25,26]. In contrast to phenylalanine concentrations, PAL enzyme activity and mRNA levels were higher earlier in tuber development (Fig. 5B and C), which is consistent with the more active phenylpropanoid metabolism observed in immature tubers.

The shikimate pathway can direct carbon flow into phenylpropanoid metabolism by providing the precursors for phenylalanine biosynthesis. Moreover, the shikimate pathway can provide additional precursors for hydroxycinnamic acid biosynthesis (Figs. 1 and 4) when partitioned toward quinate metabolism at a branch point catalyzed by the bifunctional enzyme quinate dehydrogenase, which has shikimate dehydrogenase and dehydroquinate dehydratase activity. Except for the 90 DAP samples, shikimate dehydrogenase enzyme activity, showed a trend to decreasing activity during development (Fig. 5D). Total protein in multiple genotypes was consistently about 2-fold higher in immature tubers. Consequently, when enzyme activity is normalized on a per mg protein basis, this tends to attenuate the