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Proteomic approach reveals that starch degradation contributes to anthocyanin accumulation in tuberous root of purple sweet potato

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ABSTRACT

A comparative proteomic approach was carried out to investigate anthocyanin biosynthesis in the tuberous roots of yellow sweet potato (YSP) and purple sweet potato (PSP) cultivars. More than 800 proteins were reproducibly detected through two-dimensional electrophoresis (2-DE), of which 50 proteins with 39 more and 11 less accumulated in PSP were identified through matrix-assisted laser desorption ionization-time of flight/time of flight mass spectrometry (MALDI-TOF/TOF-MS). Most of the analyzed proteins are annotated to be involved in starch metabolism and glycolysis. The more abundant starch phosphorylase (SP) and phosphoglucomutase (PGM) in PSP promoted the synthesis of precursors for anthocyanin synthesis. The results implied that starch degradation provided abundant substrates for anthocyanin biosynthesis in tuberous roots of PSP. 24 kDa vacuolar protein (VP24) is related to anthocyanin transport and accumulation in vacuoles. Vacuole-associated annexin protein, VCaB42, is correlated with tonoplast biogenesis. Synergistic action of the two proteins is probably involved in the microautophagy and the intravacuolar trapping of anthocyanins. Interestingly, both VCaB42 and VP24 were more accumulated in PSP, suggesting that anthocyanins generated in the cytosol were transported into and became stored in the vacuoles of PSP. The present study provides new insights into the mechanism of tuberous root-specific anthocyanin accumulation in PSP.

Biological significance: Sweet potato ranks as the seventh most important crop worldwide. Purple sweet potato, a special sweet potato cultivar, has been extensively investigated because large amounts of anthocyanin accumulate in its tuberous roots. Anthocyanin is well known for its free radical-scavenging activity and beneficial effects on human health. Its biosynthetic pathway has been well characterized in model plants. Although large-scale systematic studies have been performed to identify the proteins present in sweet potato, information on the regulation of anthocyanin synthesis in sweet potato is insufficient. Our proteome study demonstrated that starch degradation may contribute to anthocyanin accumulation in purple sweet potato. To our knowledge, this study is the first to propose that starch degradation may provide precursors of anthocyanin biosynthesis in sweet potato.

1. Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam.), a perennial dicotyledonous species, is an important economic food crop worldwide [1]. The tuberous root of sweet potato contains high amounts of starch, which can serve as a major carbohydrate source for human consumption, animal feed, and industrial raw material in biofuel production. Sweet potato is widely known as a high-nutrient source of dietary fiber, vitamins C, B2, B6, and E, potassium, copper, manganese, and iron; it is also a good source of low fat and cholesterol, as well as various antioxidant compounds, such as anthocyanins and carotenoids [2,3].

PSP is a special cultivar extensively investigated because of large amounts of anthocyanin accumulated in its storage roots. Anthocyanins are natural water-soluble pigments that belong to an important subgroup

Abbreviations: YSP, yellow sweet potato; PSP, purple sweet potato; 2-DE, two-dimensional electrophoresis; MALDI-TOF/TOF-MS, matrix-assisted laser desorption ionization-time of flight/time of flight mass spectrometry; SP, starch phosphorylase; PGM, phosphoglucomutase; VP24, 24 kDa vacuolar protein; VCaB42, vacuole-associated annexin protein; PAL, phenylalanine ammonialyase; CHS, chalcone synthase; DFR, dihydroflavonol reductase; F3H, flavanone-3-hydroxylase; ANS, anthocyanindin synthase; UFGT, UDP-glucose:flavonoid-3-O-glycosyl- transferase; G14, a metallothionein-like protein; AsA, ascorbic acid; DHAsA, dehydroascorbate; G-1-P, glucose-1-phosphate; GADPH, glyceraldehyde-3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; EA, enolase; 3-PGA, 3-phosphoglycerate; PEP, phosphoenolpyruvate; E-4-P, erythrose-4-phosphate; Ru-5-P, ribulose-5-phosphate; TK, transketolase; PDH, pyruvate dehydrogenase; AVIs, anthocyanic vacuolar inclusions; ABC, ATP-binding cassette transporters; MATE, multidrug and toxic compound extrusion family transporters; GME, GDP-D-mannose 3′,5′-epimerase.

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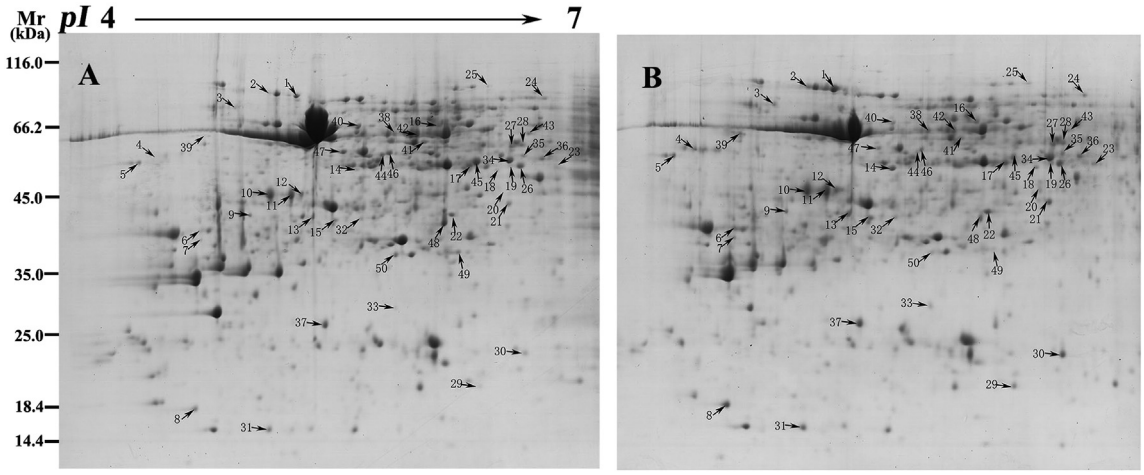

Fig. 1. 2-DE gel images of total proteins extracted from tuberous roots of two different sweet potato cultivars. Proteins were separated on IPG strips (pH 4–7), followed by SDS-PAGE on 12% polyacrylamide gels. Gels were stained with CBB. 2-D gels for YSP (A) and PSP (B). The spot numbers indicate proteins with significant difference in abundance.

of flavonoids; these pigments are of particular interest for their free radical-scavenging, anti-inflammatory, anti-cancer, and anti-diabetes activities [4–6]. Therefore, PSP with its large amount of anthocyanins in its tuberous roots can be potentially used as a component to develop drugs, such as anti-neoplastic, anti-inflammatory, and antioxidative agents [7].

The anthocyanin biosynthetic pathway has been elucidated in several plants, such as *Antirrhinum majus*, *Arabidopsis thaliana*, *Zea mays*, and *Petunia hybrida* [8,9]. Anthocyanin is synthesized via enzymatic reactions in the cytoplasm and endoplasmic reticulum membrane; then transported and accumulated in vacuoles or cell wall. Anthocyanin biosynthesis can be regulated by internal factors, including MYB, helix–loop–helix (bHLH), and WD40 protein [10]. Anthocyanin biosynthesis is also modulated by external factors, such as temperature [11], light [12], plant growth regulator [13,14], and sugars [15].

There are several reports about the anthocyanin biosynthesis regulated by sugars. Up-regulated anthocyanin accumulations in sugar treatment condition were observed in *Arabidopsis*, radish hypocotyls and grape berries [16–18]. Several genes, including dihydroflavonol reductase (*DFR*), leucoanthocyanidin dioxygenase (*LDOX*), and flavanone-3-hydroxylase (*F3H*), regulated by sucrose were found in grape berries [19–21]. Moreover, Solfanelli et al. [22] conducted further research on the regulation of anthocyanin in *Arabidopsis* treated with sucrose and found that sugar could regulate anthocyanin biosynthesis via a sucrose-specific pathway. And signal molecules, such as Ca^{2+}, protein kinases, and phosphatases, can participate in the sugar-inducted regulation of anthocyanin [23].

Recently, some large-scale systematic studies have been conducted in sweet potato. Lee et al. [24] compared the protein expression levels between two sweet potato cultivars, and identified several proteins related to nematode resistance. Shubhendu et al. [25] analyzed the nutrient availability of two sweet potato cultivars and suggested that genotypic variability may explain the cultivar-specific regulation of nutrient production. However, information regarding the regulation of anthocyanin synthesis in PSP is insufficient. In our study, 2-DE and MALDI-TOF/TOF-MS were employed to investigate the regulation of anthocyanin synthesis. These results may help enhance our understanding of the mechanisms of anthocyanin accumulation in the PSP cultivar.

2. Materials and methods

2.1. Plant materials

Two sweet potato cultivars (Zishu 404 and Huangshu 8) were grown in the test field of the Fujian Vocational College of Agriculture in Fuzhou city, China. The tuberous roots of sweet potato were harvested in the rapid growth period (40 days after planting). For each cultivar, 9 tubers with a central diameter of 4–6 cm from 9 different plants were harvested respectively, and then divided into three independent biological replicates. After that, every 3 tubers were peeled, sliced in liquid nitrogen and mixed completely. Finally, the pooled mixture was stored at −80 °C for further protein and RNA extraction.

2.2. Preparation of total protein extraction

The total proteins of the tuberous roots were extracted via ethanol precipitation method in accordance with the methods described by Lee et al. [26], who prepared a depletion method for abundant storage proteins, such as sporamin in sweet potato, to determine low-concentration proteins in proteome research. In brief, the tuberous roots were ground in precooled extraction buffer I, which contained 100 mM KCl, 100 mM Tris–HCl (pH 8.0), 50 mM disodium tetraborate decahydrate, 2% Polyvinyl-pyrrolidone, 1 mM phenylmethylsulfonyl fluoride, 0.5% (*v*/*v*) Triton X-100, 50 mM L-ascorbic acid (AsA), and 2% (*v*/*v*) β-mercaptoethanol. After homogenization was completed, the supernatant was mixed with equal volumes of 100% ethanol. Ethanol was used to separate the supernatant and pellet fractions after centrifugation was performed. Then the supernatant was discarded, the pellet fraction was resuspended in extraction buffer I and homogenized with an equal volume of cooled Tris-saturated phenol (pH 8.0). After centrifugation, equal volumes of extraction buffer II (extraction buffer I with 0.7 M sucrose) was added to the low phenol phase. Afterward, 100 mM cold ammonium acetate/methanol precipitation was performed. The sample was washed with acetone, dried at room temperature, and stored at −80 °C.

Notes to table 1
[a] Numbering corresponds to the 2-DE gel in Fig. 1.
[b] The number of the predicted protein in NCBInr.
[c] Molecular mass and *pI* theoretical and experimental.
[d] Percentage of predicated protein sequence with matched sequence.
[e] Y, yellow sweet potato; P, purple sweet potato.
[f] The abundance ratios of each spot were compared between YSP and PSP.

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