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In Situ and In Vitro Antioxidant Activity of Sweetpotato Anthocyanins

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Anthocyanins from a variety of fruits and vegetables have been shown to possess potent antioxidant activity in vitro, but scavenging of free radicals by anthocyanins has only been demonstrated in situ in the leaves of certain plants. We report on a new sweetpotato that exhibits mottled purple flesh attributable to high concentrations of anthocyanins. By perfusing transverse sweetpotato sections with the reactive oxygen species H_2O_2, followed by the H_2O_2 sensitive fluorochrome scopletin, we show that anthocyanins act as antioxidants in situ within the sweetpotato storage roots. We also demonstrate in vitro antioxidant activity by sweetpotato anthocyanins, where an additive effect with hydroxycinnamic acids is observed. Anthocyanic foods have been shown to offer protection against a variety of degenerative disease processes. Given that sweetpotato can be eaten several hundred grams at a time and as a staple, these data are consistent with the possibility of superior health protection by anthocyanic varieties of sweetpotato in comparison to most common fruits and vegetables.

KEYWORDS: Anthocyanin; antioxidant; *Ipomea batatas*; sweetpotato

INTRODUCTION

Sweetpotato, *Ipomoea batatas* (L.) Lam., is grown in 111 countries, 90% of which are classified as "developing countries", and ranks as the seventh most important food crop after wheat, rice, maize, potatoes, barley, and cassava (*1*). Sweetpotato is nutritionally valuable, with higher levels of both carbohydrate and dietary fiber than potato (*Solanum tuberosum*) (*2*), and a strong antioxidant activity that has been claimed to surpass most other vegetables in a typical Western diet (*3*). Hydroxycinnamic acids (HCA) are the main phenolic antioxidants in most commercially available sweetpotato varieties (*4*), which can vary in storage root size, shape, flavor, texture, and color, with the most common being white-, cream-, yellow-, or orange-fleshed. In Japan and New Zealand, several varieties of sweetpotato have been developed with intense purple coloration, conferred by high anthocyanin content, including clone 97D, which arose as a spontaneous mutation of the commercial cultivar Toka Toka Gold (TTG) (Andre de Bruin, Delta Produce Ltd., personal communication) (**Figure 1A**,**B**).

Anthocyanins are flavonoid compounds responsible for the red/blue coloration of many fruits and flowers. Anthocyanins from sources such as blueberries, strawberries, red wine (*5*), and sweetpotato (*6*−*9*) have been shown to be potent antioxidants in vitro, exhibiting greater antioxidant activity than either vitamin C or vitamin E. The primary function of anthocyanins in fruits and flowers is suggested to be as animal attractants rather than antioxidants, encouraging seed dispersal or pollination (*10*), whereas in leaves or structures such as sweetpotato storage roots, anthocyanins have been suggested to play a role in defense response and protection from herbivory (*11*). Interestingly, it has been demonstrated that anthocyanin biosynthesis is induced or up-regulated in the leaves of many plants following a variety of environmental and biotic stresses that are known to cause the formation of reactive oxygen species (ROS) and that red leaves possess greater antioxidant activity than green leaves in *Elatostema rugosum* (*12*). Furthermore, comparison of the oxidative burst associated with mechanical injury in red and green portions of *Psuedowintera colorata* leaves using the fluorochromes dichlorofluorescein and scopoletin demonstrated that anthocyanins can act as antioxidants in situ in leaves (*13*). To date, however, in situ antioxidant activity by anthocyanins has not been demonstrated in the edible portions of a food plant.

Here, we use the scopoletin fluorochrome to investigate the potential for anthocyanins to protect against ROS in sweetpotato storage roots in situ, and we correlate anthocyanin content of the storage roots to in vitro antioxidant activity.

MATERIALS AND METHODS

Sweetpotatoes. 97D arose as a spontaneous mutation from TTG. Sample roots of both clones were provided by Delta Produce Ltd., Dargaville, New Zealand.

In Situ Measurement of Free Radicals. Transverse sweetpotato tissue sections approximately 10 μm thick were cut by a vibratome and perfused with 5 mM H_2O_2 in Tris-KCl buffer under fluctuating

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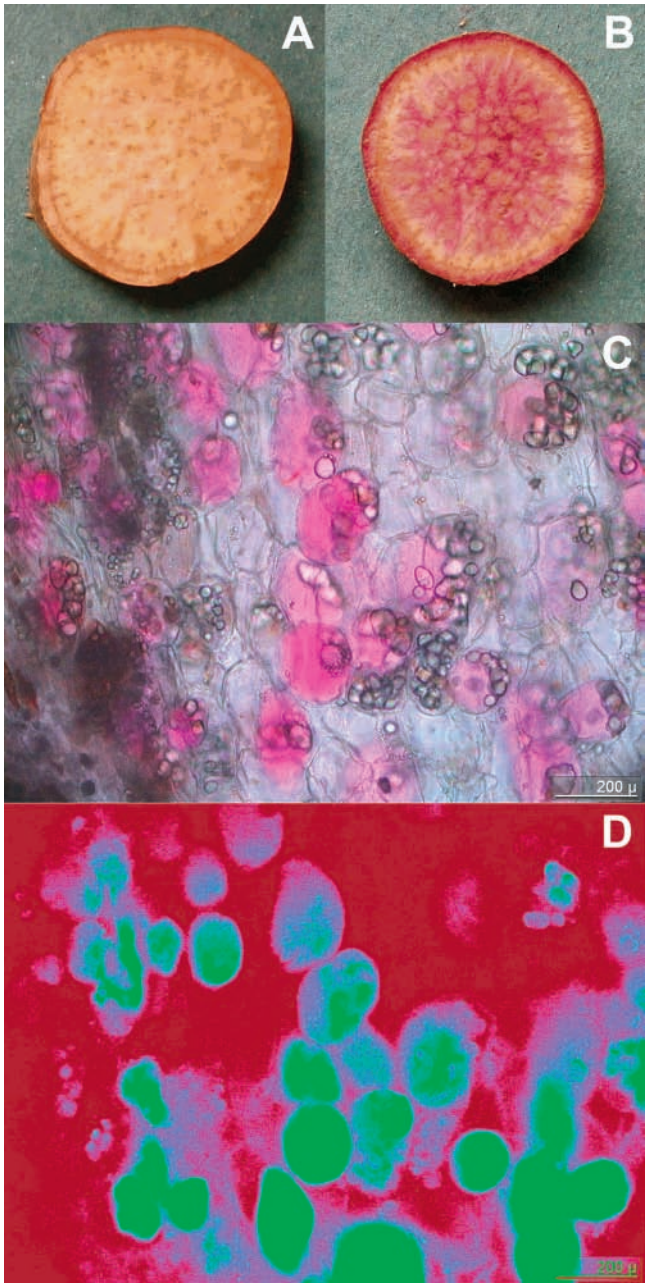

Figure 1. (**A**,**B**) Coloration in the 97D mutant (**B**), due to anthocyanin content, in comparison to the wild-type TTG (**A**). (**C**,**D**) In situ free radical scavenging by anthocyanic cell in 97D storage roots. (**C**) Bright field image showing interspersed anthocyanic (pink) and acyanic (clear) parenchymal cells. (**D**) False color image of H_2O_2 concentration as indicated by the fluorochrome scopoletin; red areas indicate high H_2O_2 concentrations, and green areas indicate low H_2O_2 concentrations. Red areas correspond to acyanic cells, while green areas correspond to anthocyanic cells of the bright field image.

pressure. Sections were washed three times in buffer and perfused with 100 mM scopoletin in buffer under fluctuating pressure. After the sections were rinsed in buffer, they were mounted on slides and viewed at 20× magnification, first in bright field and then under fluorescent light (excitation 365 nm).

Sweetpotato Extracts and Anthocyanin Concentration. Sweetpotato extracts were made by homogenizing 10 g of flesh in 100 mL of 7% acetic acid in methanol. Anthocyanin concentration was determined by dilution of samples in 10% HCl in methanol followed by measurement of absorbance at 527.5 nm and calculation of anthocyanin content using Beer's law, with an extinction coefficient (ϵ) of 33 000 (cyanidin-3glucoside).

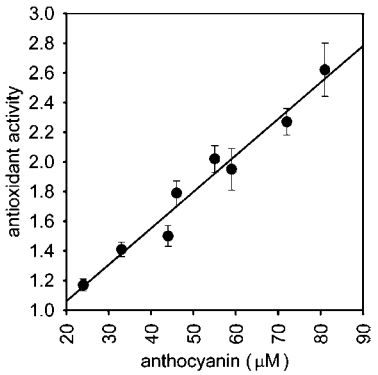

Figure 2. Correlation between anthocyanin content and antioxidant activity (expressed as mM ascorbic acid equivalents) of methanolic extracts from eight 97D storage roots. Vertical lines represent the standard error of the mean of duplicate samples (adjusted $R^2 = 0.9598$, $p < 0.001$).

Measurement of Antioxidant Activity. Duplicate 1:2 serial dilutions of samples or 1 mM ascorbic acid standard were made across the wells of 96 well plates. Wells contained volumes equivalent to 50−0.05 μL of diluted sample or standard with the last row containing extraction solution only. A 150 μL amount of 100 μM 1,1-diphenyl-picrylhydrazyl (DPPH) in methanol was added to all wells, and the plates were allowed to stand for 30 min before absorbances were read at 515 nm. Duplicates were averaged, and the volume of sample or standard required to halve DPPH absorbance was calculated (IC_{50}). Antioxidant activities were expressed relative to the standard as mM ascorbic acid equivalencies.

RESULTS AND DISCUSSION

Anthocyanins from a variety of fruits have been shown to exhibit potent antioxidant activity in vitro (*5*), although it is unclear whether they function in this capacity in fruit in situ. Recently, however, in situ scavenging of ROS by anthocyanins has been described in the leaves of *Psuedowintera colorata* (*13*). Here, we have demonstrated that anthocyanins can also protect from ROS in the edible storage roots of purple sweetpotato. Transverse sections through the parenchyma of the 97D sweetpotato exhibited a combination of anthocyanic (pink) and acyanic (clear) cells (**Figure 1C**). Perfusion of the ROS H_2O_2 into the cells followed by scopoletin, which fluoresces in the presence of H_2O_2, demonstrated that H_2O_2 was rapidly inactivated in anthocyanic cells (**Figure 1D**).

Anthocyanins are produced in leaves in response to various stresses, including wounding (*14*), UV light (*15*), and pathogen attack (*11*). These stress responses involve the generation of ROS, and anthocyanins may be produced as a secondary response to protect plant tissue from free radical damage. The sweetpotato 97D occurred as a spontaneous mutant of the acyanic TTG, suggesting that the mutation occurred in the anthocyanin biosynthetic pathway or in a stress signaling pathway, resulting in increased anthocyanin production even in the absence of external stress signals.

Antioxidant activity in extracts from acyanic sweetpotato has previously been attributed to HCA content (*4*). Extracts from eight 97D storage roots showed considerable variability in anthocyanin concentration, but when antioxidant activity of the extracts was measured, there was a clear linear relationship between anthocyanin concentration and antioxidant activity (**Figure 2**). However, anthocyanins could not be the sole antioxidants present as extrapolation of the data predicts significant antioxidant activity even in the absence of anthocyanins. Isolation of individual components of the 97D extracts by the paper chromatography methodology of Markham and Bloor (*16*), followed by measurement of their antioxidant

activity in the DPPH assay, showed that this background antioxidant activity was attributable to HCAs and that any increases in antioxidant activity were the result of an additive effect with anthocyanins (data not shown).

The potent antioxidant activity of anthocyanins is thought to play a major role in the beneficial health effects of anthocyanins, which include maintenance of normal vascular permeability (*17*), vasoprotective and antiinflammatory properties (*18*), protection against cancer (*19*), and prevention of neural decay during the aging process (*20*). Foods such as blueberries are often promoted as dietary sources of anthocyanins, containing 2.67 ± 0.097 μmol/g (*21*). We have previously described sweetpotato cultivars with anthocyanin contents as high as 4.02 ± 0.095 mmol/g (*6*). Furthermore, whereas the highest per capita consumption of blueberries is in the U.S. at 0.33 kg/year (*22*), sweetpotato is consumed in excess of 100 kg/person/year in some countries (*23*). Therefore, given that sweetpotato is consumed in larger portions and as a staple, there is potential for greater anthocyanin intake, and consequent benefit, from anthocyanic sweetpotato than from other sources.

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