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Characterization of Anthocyanins in Sweet Potato Leaves Grown in Various Stages and Conditions

Xiaoyu Su[1], Zhenbao Jia[2], Fei Tao[2], Jiamin Shen[1], Jingwen Xu[1], Jason Griffin[3] and Weiqun Wang[1*]

[1]Department of Food Nutrition Dietetics and Health, Kansas State University, Manhattan, KS 66506, USA.
[2]Department of Food Quality and Safety, China Jiliang University, Hangzhou, Zhejiang 310018, China.
[3]Department of Horticulture and Natural Resources, Kansas State University, Manhattan, KS 66506, USA.

Authors' contributions

This work was carried out in collaboration among all authors. Authors JG and WW designed the study. Author JG performed sweet potato growth. Author XS wrote the first draft of the manuscript. Authors ZJ, FT, JS and WW finally edited the reversion. All authors read and approved the final manuscript.

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ABSTRACT

Phytochemical-enriched edible greens, sweet potato leaves (*Ipomoea batatas* L.), have become popular due to potential health benefits. However, the phytochemical contents in sweet potato leaves and their subsequent change over harvest stages and growth condition are mostly unknown. In this study, the anthocyanin profile and content in leaves of four sweet potato cultivars, i.e., white-skinned and white-fleshed Bonita, red-skinned and orange-fleshed Beauregard, red-skinned and white-fleshed Murasaki and purple-skinned and purple-fleshed P40, were evaluated. Fourteen anthocyanins were isolated and identified by HPLC-MSI/MS. The most abundant was cyanidin 3-caffeoyl-p-hydroxybenzoyl sophoroside-5-glucoside, which comprised up to 20% of the total anthocyanins. Of the young leaves (1st and 2nd slip cuttings), Bonita contained the highest anthocyanin content followed by P40. Of the mature leaves (vine stage), Beauregard had the

**Corresponding author: Email: wwang@ksu.edu;*

greatest anthocyanin (592.5 ± 86.4 mg/kg DW) and total phenolic (52.2 ± 3 mg GAE/g DW). It should be noted that the lowest anthocyanin and total phenolic content of shoots were found in P40, while tubers of P40 contain the highest content of each. Furthermore, the increase in leaf anthocyanin content over the growth stages that was observed in three of the cultivars but not in P40. No significant difference of anthocyanin content was found in Beauregard leaves grown in the high tunnels when compared with that in the open field. This study demonstrated for the first time that anthocyanin levels were significantly changed in response to various growth stages but not high tunnel condition, indicating that the effect of anthocyanin biosynthesis in sweet potato leaves is highly variable and genotype specific.

Keywords: Anthocyanins; growth stages; HPLC-ESI/MS; sweet potato leaves.

1. INTRODUCTION

Sweet potato (*Ipomoea batatas* L.), one of the six most important food crops in the world, is cultivated in many developing countries. [1] This well-known crop is highly productive although it requires low input [2]. The tubers of the sweet potato are nutritious and commonly consumed, but sweet potato generates many by-products like leaves, residue and waste water. Sweet potato leaves have been consumed as a green leaf vegetable in African and Asian countries and contain protein, essential amino acids, antioxidants, vitamin B, minerals and dietary fiber. [3-6] Both purple and green sweet potato leaves had been reported at the high levels of phytochemicals and other bioactive compounds that are excellent sources of anti-oxidative polyphenols like anthocyanins and phenolic acids [7-9].

Anthocyanins are the primary subclass of polyphenols in the red, purple and blue pigmentation of many plants. They represent a diverse group of secondary metabolites in higher plants [10]. Over past few years, anthocyanins in sweet potato were paid much attention due to their numerous beneficial activities. Islam et al. demonstrated that sweet potato leaves were an excellent source of anti-oxidative polyphenols like anthocyanins and phenolic acids, which were widely used as functional food ingredients that enhanced the antioxidant capacity of consumed food products in the U.S. food market. 3 Additionally, the findings in experimental animal model have demonstrated that anthocyanin-rich purple sweet potato P40 may protect against colorectal cancer by inducing cell-cycle arrest, anti-proliferation, and apoptotic induction [11,12].

Phytochemical content depends not only on genotype but also on growth environment. Fluctuations in light, water stress, temperature, rain intensity and air humidity restrict nutritive components and could also affect plant phytochemical content and quality [13-17]. For example, high tunnel growth chambers provide an easy, cost-effective way to maintain stable growth conditions and extend the sweet potato growth season, which enhances yield and profitability. Growth stage is yet another source of variability that influences secondary metabolite concentration [16,17]. Therefore, determining optimum growth stages and growth conditions for sweet potato leaves should maximize anthocyanin content (AC) and total phenolic content (TPC), contributing to assessing sweet potato leaves as feasible, functional food [18].

No report has been published on the differences in AC and TPC at different sweet potato leaf development stages. Few studies have been conducted on how high tunnel and open field growth conditions affect AC and TPC of sweet potato leaves. This study was set up to investigate the effect of various growth stages and conditions on anthocyanin accumulation in the leaves of four sweet potato varieties: White-skin and white-fleshed Bonita, red-skin and orange-fleshed Beauregard, red-skin and white-fleshed Murasaki and purple-skin and purple-fleshed P40. The results would provide anthocyanin profiles and content in different varieties of sweet potato leaves in response to various growth stages and high tunnel conditions.

2. MATERIALS AND METHODS

2.1 Materials

Acetonitrile, methanol, and formic acid used in this study were either HPLC grade or analytic grade and purchased from Thermal Fisher Scientific (Suwanee, GA, USA). Water was purified through Barnstead E-Pure Deionization System (Dubuque, IA, USA) and filtered through Millpore 0.45 μm membrane (Bedford, MA,

USA). A standard of Peonidin-3-glucoside chloride, Folin-Ciocalteu reagent and gallic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Sample Preparation and Extraction

The leaves of four sweet potato cultivars (Bonita, Beauregard, Murasaki, and P40) were harvested at the Kansas State University, John C. Pair Horticultural Center. The 1st sweet potato slips were removed from tubers at two weeks of the growth cycle, and the 2nd sweet potato slips at six weeks. The vine stage came from 2nd sweet potato slips that were cut and re-planted for an additional six weeks of growth. For each variety, leaves were washed with tap water, chopped into approximately 2 cm slices, freeze-dried (Labconco, Free Zone 2.5) and ground by a food processor into powder. Prepared powder was then stored at -80ºC until further extraction. To prepare anthocyanin extracts, 0.5 g of powder was extracted with 20 mL of mixed solvent of water and 1 N formic acid at 95:5 (v/v). Tubes containing powder/solvent mixture were wrapped with aluminum foil to avoid light exposure. After a 24h extraction, the suspensions were centrifuged at 4000 g for 45 min, and the supernatant was collected and dried by vacuum drier at 25ºC overnight. Subsequently, 1 mL of acidified methanol was added, and then the dissolved extract was filtered through a Whatman syringe filter (Whatman 0.45µm PVDF) for HPLC-MS/MS analysis.

2.3 Identification and Analysis of Anthocyanins by HPLC-MSI/MS

HPLC coupled Electrospray Ionization tandem Mass Spectrometry (HPLC-MSI/MS) was used to carry out anthocyanin identification and quantification according to our previous publications [8,11,19]. A Shimadzu HPLC system (Kyoto, Japan) was used for chromatographic analysis and separation. This system used a DGU-20A3 built-in degasser, a LC-20AB solvent delivery pump, a SIL-20ACHT auto-sampler, a CTO-20AC column holding oven, a CBM-20A communicator module and a SPDM20A Photodiode Array Detector. A Waters (Milford, MA, USA) C18 reversed phase column (250 × 4.6 mm) was used for anthocyanin separation. Elution was performed with mobile phase A (5% formic acid in de-ionized water) and mobile phase B (5% formic acid in acetonitrile/water 1:1 v:v). The gradient conditions were set as follows: solvent B volume at 20-40% for 30 min, 40-50% for the following five min, held at 50% for 10 min, before returning to 20%. The detector performed a full spectrum scan between 190 and 800 nm, but 520 nm was used for monitoring anthocyanins. Peonidin-3-glucoside was used as an internal standard for quantitation of extraction recovery, and AC was expressed as peonidin 3-glucoside equivalent (PN3GE) per g dry weight (DW). Based on a signal-to-noise ratio of 3:1 and the standard deviation of the lowest concentration of PN3G/slope of the calibration line, the detection limit was estimated at 5 µmol.

Mass spectrometric scan was performed on a Bruker Esquire 3000 in positive mode with a scanning interval 500-1200 m/z. Data were analyzed using Bruker Hystar Post Processing software (Bruker Daltonics, GmbH, Billerica, MA, USA). The ESI/MS data was used to confirm the mass of each anthocyanin HPLC peak. The mass spectrometry instrument was controlled by the esquire control 5.3 software (Bruker Daltonics, GmbH, Billerica, MA, USA) and the data were processed with Data analysis 3.3 software (Bruker Daltonics, GmbH, Billerica, MA, USA). Each anthocyanin was individually identified by comparing HPLC retention time, absorbance spectra, and MS spectra with previously published works [11,19]. New anthocyanin's mass spectral data were matched by the National Institute of Standards and Technology Mass Spectra Library data (NIST08, National Institute of Standards and Technology, Gaithersburg, MD, USA).

2.4 Measurement of TPC

TPC in each sweet potato extract were measured by Folin-Ciocalteu method with a slight modification [20]. A stock solution of 1mg/mL Gallic acid in distilled water was prepared ranging from 12.5-200 µg/mL in 70% acetone for the standard curve. A solution of Na_2CO_3 at 7.5% (w/v) was also prepared. To each of the 96 wells, 75 µL of distilled water was added, followed by 25 µL of either aliquots of extracts or gallic acid standard at various concentrations. Folin-Ciocalteu regent diluted 1:1 with distilled water was then added to each well. The wells then stood for 10 min at room temperature before 100µL of Na_2CO_3 solution was added to each well. Plate was covered and stood in darkness for 90min before measuring. Absorbance was read in a microplate reader Synergy HT, biotek (winnoski, USA) using Gen5™2.0 data analysis software. Results were expressed as mg Gallic acid equivalent (GAE) per g dry weight (DW).

2.5 Statistical Analysis

Data were analyzed using SAS statistical software, version 9.3 (SAS Institute, Cary, NC, USA). Data were analyzed by overall two-way ANOVA followed by Tukey's test for individual between-group comparisons. The results were presented as means ± SD, and a probability at $p \leq 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

3.1 Chromatographic Separation

Chemically, anthocyanins are a group of flavonoids characterized by having C_6-C_3-C_6 skeleton. Among the more than 600 types of anthocyanin, [21] most anthocyanin aglycones found in nature comprise six anthocyanidins: cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin, each with a 2-phenylbenzopyrilium (flavyl-ium) skeleton hydroxylated in the 3, 5 and 7 positions, with different substitutions at R_1 and R_2 (Fig. 1). Unlike other flavonoids, anthocyanins have a positive charge on the C-ring, which responds to pH with different colors [11]. The objectives of this study were to use HPLC-MSI/MS to characterize the anthocyanin profile and quantify the anthocyanin content of sweet potato leaves in response to various growth stages and conditions. Fig. 2 shows the profile of anthocyanin peaks from sweet potato leaves in HPLC chromatogram. No anthocyanins were detectable in the stem of P40, Murasaki, Bonita, and Beauregard, but fourteen anthocyanins, including one newly discovered, were found in the sweet potato leaves at retention times between 4 and 27 min. Of these, peaks 8 and 9 were the major anthocyanins and their peak areas appeared to cover more than half of total anthocyanin peak areas.

3.2 Mass Spectrometric Identification

Following HPLC separation, LC-MSI/MS data were characterized by monitoring the molecular ion characteristics for each peak. Table 1 provides the *m*/*z* ratio of each intact anthocyanin and its fragment ions. As shown in Table 1, Cyanidin (Dpd *m*/*z* 287) and Peonidin (Ptd *m*/*z* 301) were the two anthocyanidin aglycones detected in all four varieties of sweet potato leaves. Thirteen of the fourteen anthocyanins including Cyanidins (peaks 1, 3, 4, and 7-10) and Peonidin (peaks 2, 5, 6 and 11-13) have been reported by Islam, et al. [7] However, one new Peonidin (peak 14) was found in the all four varieties for the first time. The m/z ratio of a molecular ion with a fragment ion was captured within the scanning interval of 500–1200 m/z. The ions for peak 14 (Peonidin 3-caffeoyl-p-coumaryl sophoroside-5-glucoside; *m*/*z* 1095), produced three fragments at *m*/*z* 933, 463, and 301. Transition 1095-933 implied a loss of glucose (*m*/*z* 162), and 1095-463 a loss of 3-caffeoyl-p-coumaryl sophoroside (*m*/*z* 632). Transition 1095-301 produced Peonidin aglycone (*m*/*z* 301) caused by the loss of glucose and 3-caffeoyl-p-coumaryl sophoroside.

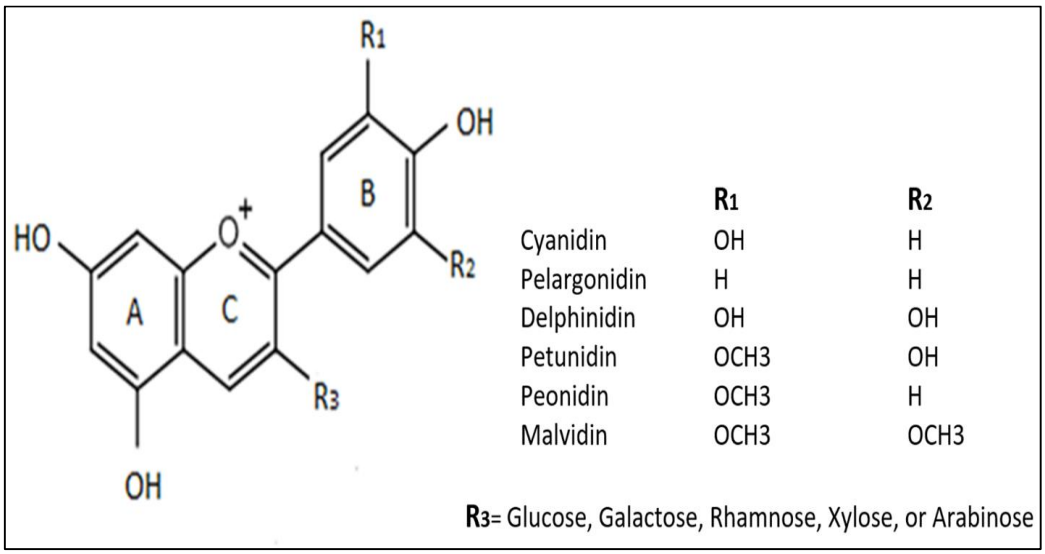

Fig. 1. Chemical structures of common anthocyanidins and anthocyanins

Fourteen anthocyanins were isolated and identified in all four varieties of sweet potato leaves. Although this new anthocyanin could be detected in purple sweet potato tubers (P40), [22] previous studies had not found Peonidin 3-caffeoyl-p-coumaryl sophoroside-5-glucoside in any sweet potato leaves; this new anthocyanin was first reported in Murasaki sweet potato leaves.

3.3 AC and TPC in Sweet Potato Leaves

AC at both slip and vine stages of all four varieties of sweet potato leaves is given in Fig. 3. No significant variation across the four varieties was found at the 1st slip stage. Results showed that Bonita produced the most AC during 1st and 2nd slip stages (280±30 mg /kg; $p<0.05$) while Beauregard had the richest AC at vine stage (592.5±86.4 mg/kg; $p<0.05$), reaching 592.5±86.4 mg/kg of the extracts, approximately 10 times more abundant than the amount of AC at 2nd slip stage. AC increased continuously in Bonita from 1st slip stage to vine stage, but P40 did not. Moreover, Bonita also showed high AC at vine stage, with 473.9±79.9 mg/kg, significantly more than Murasaki and P40 leaves at the same stage ($p<0.05$). The changes in AC at P40 vine stage did not correspond to the changes observed in the other varieties. AC of P40 even decreased significantly from 2nd slip to vine stage.

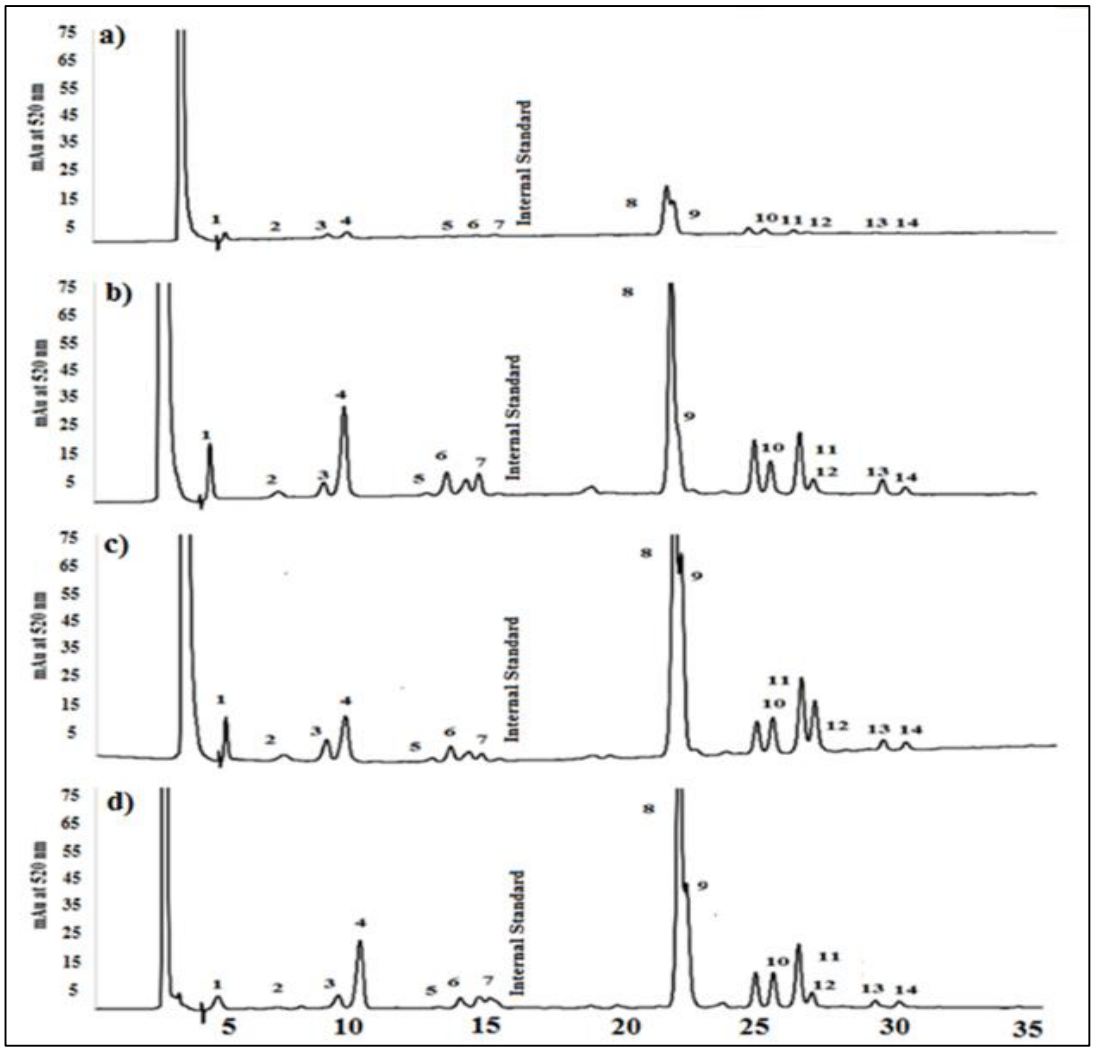

Fig. 2. Representative HPLC chromatograms of anthocyanins in sweet potato leaves of vine stage: a) P40; b) Murasaki; c) Bonita; d) Beauregard (The peak number corresponding to anthocyanin name is shown in Table 1)

Table 1. Mass spectrometric data of Anthocyanins in sweet potato leaves

Peak number	Retention(min)	Anthocyanins	[M+H] (m/z)	Fragment ions(m/z)
1	3.61	Cyanidin-3-sophoroside-5-glucoside	773	661,449,287
2	5.28	Peonidin-3-sophoroside-5-glucoside	787	595,433,271
3	6.23	p-hydroxybenzoylated (Cyanidin 3-sophoreside-5-glucoside)	893	731,449,287
4	6.96	Caffeoylated (Cyanidin 3-sophoroside-5-glucoside)	935	773,449,287
5	9.75	p-hydroxybenzoylated (Peonidin 3-sophoroside-5-glucoside)	907	745,463,301
6	10.29	Caffeoylated (Peonidin 3-sopheroside-5-glucoside)	949	787,463,301
7	11.31	Feruloylated (Cyanidin 3-sophoroside-5-glucoside)	949	787,449,287
8	17.90	Cyanidin 3-(6,6'-dicaffeoyl sophoroside)-5-glucoside	1097	935,449,287
9	17.57	Cyanidin 3-(6,6'-caffeoyl-p-hydroxybenzoyl sophoroside)-5-glucoside	1055	893,449,287
10	21.26	Cyanidin 3-(6,6'-caffeoyl-feruloyl-sophoroside)-5-glucoside	1111	949,449,287
11	22.23	Peonidin 3-(6,6'-dicaffeoyl-sophoroside)-5-glueoside	1111	949,463,301
12	22.85	Peonidin 3-(6,6'-caffeoyl-p-hydroxybenzoyl sophoreside)-5-glucoside	1069	907,463,301
13	25.30	Peonidin 3-(6,6'-caffeoyl-feruloyl sophoroside)-5-glucoside	1125	963,463,301
14	26.09	Peonidin 3-caffeoyl-p-coumaryl sophoroside-5-glucoside	1095	933,463,301

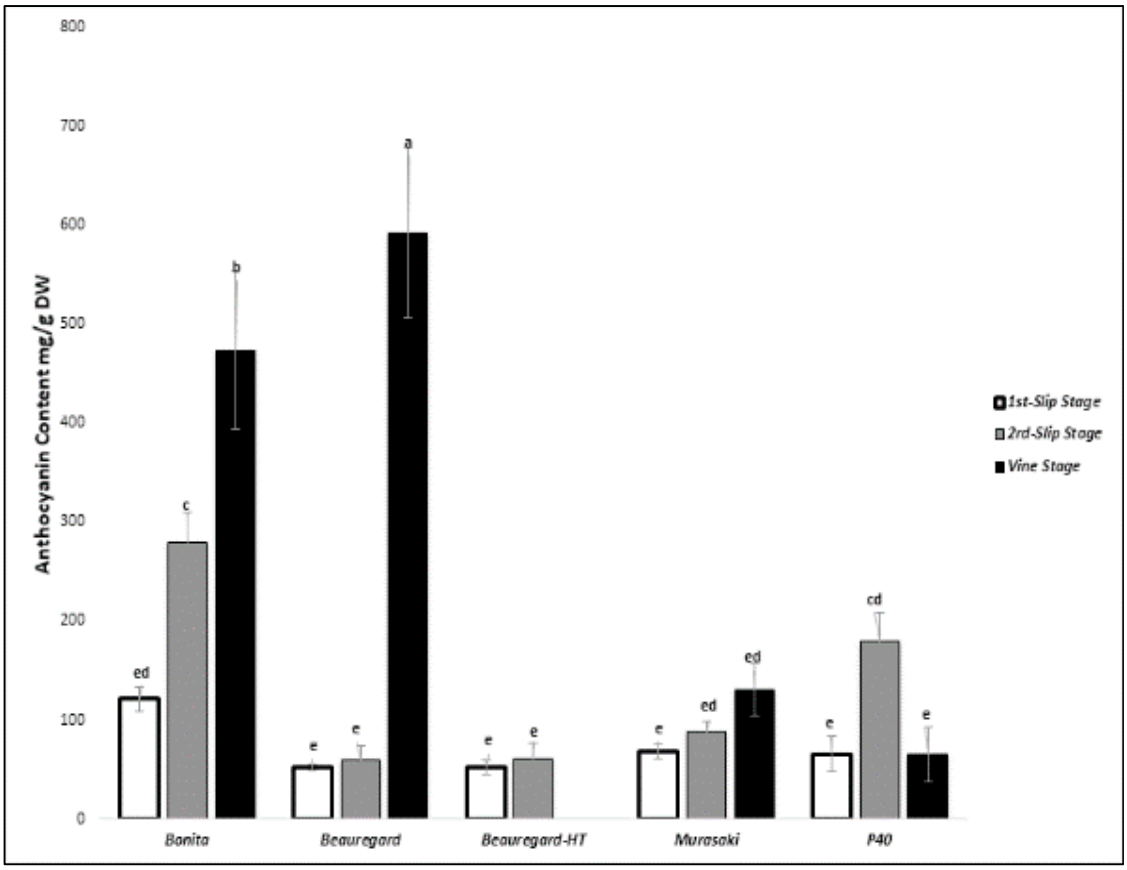

Fig. 3. Anthocyanin content of Bonita, Beauregard, Beauregard high tunnel (Beauregard HT), Murasaki and P40 leaves under three different growth stages: 1st slip stage, 2nd slip stage and vine stage. Bars represent mean ± SE (n=4), Means in a cluster with the same letters are not significantly different at the 0.05 level

The predominant anthocyanins were Cyanidin3-(6, 6'-dicaffeoyl-sophoroside)-5-glucoside, Cyanidin3-(6, 6'-caffeoyl-p-hydroxybenzoyl sophoroside)-5-glucoside, up to 60% of total anthocyanins within slip and vine stages. The new anthocyanin, Peonidin 3-caffeoyl-p-coumaryl sophoroside-5-glucoside accounted for approximately 1% of total anthocyanins. As reported by Xum et al. [19] that the top three main anthocyanins in P40 flesh were Cyanidin3-(6,6'-caffeoyl-p-hydroxybenzoyl sophoroside)-5-glucoside, peonidin 3-caffeoyl sophoroside-5-glucoside, and Cyanidin3-(6,6'-caffeoylferuloyl-sophoroside) -5-glucoside, which accounted for half the total AC. Of these, Cyanidin 3-p-hydroxybenzoyl sophoroside-5-glucoside showed high levels in both flesh and leaves of P40.

We found no significant changes in TPC in Murasaki from 1st slip stage to vine stage ($p<0.05$) (Fig. 4); Beauregard at vine stage had the most abundant TPC (52.2±3 mg GAE/g DW), followed by Murasaki (44.6±2.4 mg GAE/g DW) and Bonita (40.8±3.3 mg GAE/g DW) ($p<0.05$) (Fig. 4). Please note that TPC in P40 leaves did not change significantly for any stage, ranging from 36.3 ±1 mg GAE/g DW at 1st slip to 34.5 ± 1.4 mg GAE/g DW at vine stage. It should be noted the contents of anthocyanins and other phytochemicals in response to various growth stages are important not only for sweet potato breeders but also for the consumers as they relate to potential health benefits.

In the field experiment, AC of Beauregard at slip stages did not differ significantly between high tunnel and open field environments ($p<0.05$). Similarly, TPC for Beauregard slip stages grown in the field was not higher than the high tunnel, indicating that Beauregard slips in high tunnels may not provide impact on phytochemical

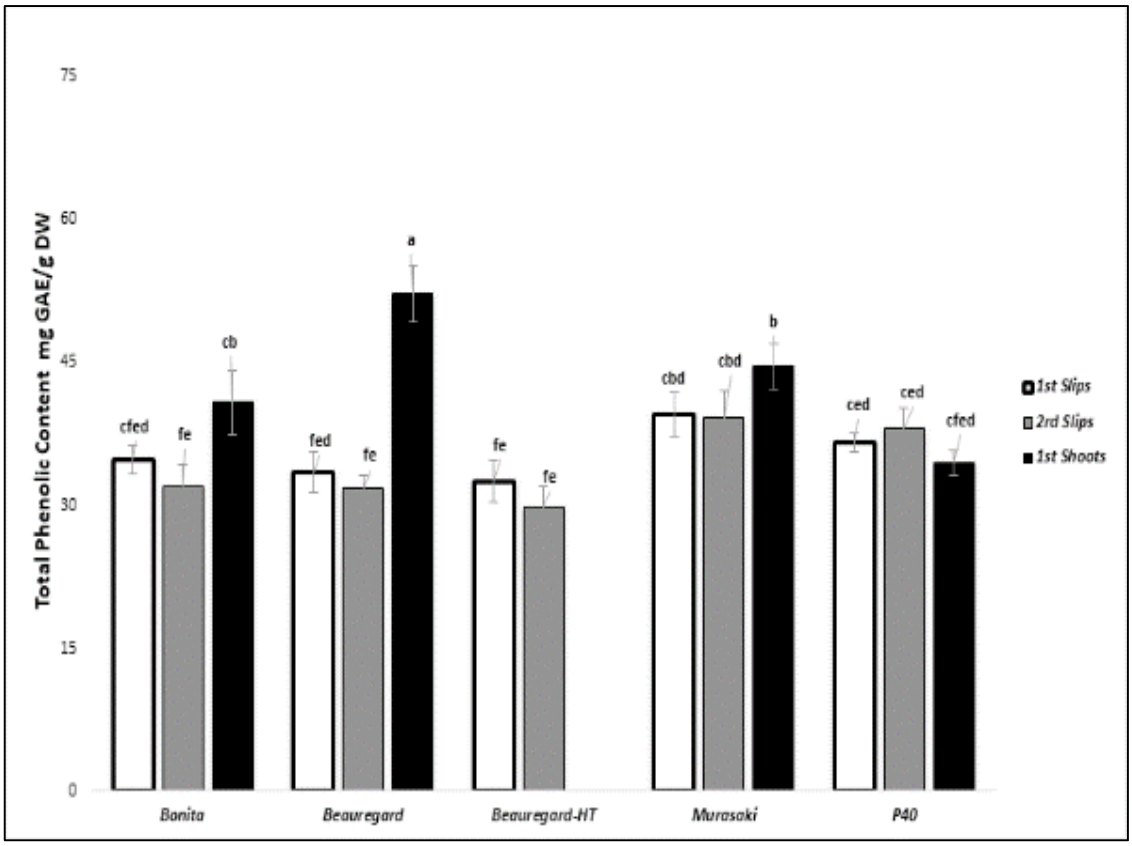

Fig. 4. Total phenolic content of Bonita, Beauregard, Beauregard high tunnel (Beauregard HT), Murasaki and P40 leaves under three different growth stages: 1st slip stage, 2nd slip stage and vine stage. (Bars represent mean ± SE (n=4), Means in a cluster with the same letters are not significantly different at the 0.05 level)

biosynthesis directly. It has been reported that polyphenols like anthocyanins are sensitive to adverse environmental conditions, including unfavorable temperature, light, pH and humidity, so growing sweet potatoes in a high tunnel does provide an easy and cost-effective way to establish more control over the growing environment and extend the growing season to enhance crop yield, quality, and profitability. [23] Despite the potential benefits of using the high tunnel at 1st and 2nd slip stages, additional study could determine the extent to which the vine stage might contribute to differing AC and TPC in the high tunnel condition.

4. CONCLUSION

In conclusion, fourteen anthocyanins, including one new one, were identified and quantified by HPLC-MSI/MS in the white-skin and white-fleshed Bonita, red-skin and orange-fleshed Beauregard, red-skin and white-fleshed Murasaki and purple-skin and purple-fleshed P40. This study showed for the first time that anthocyanin levels are significantly affected by growth stage. Although the P40 tubers had the highest content of anthocyanins, Beauregard leaves at vine stage were richest in AC and TPC among the studied samples. AC increased continuously in Bonita from 1st slip stage to vine stage, but P40 did not have the same response. In the high tunnel studies, no significant differences in AC and TPC were found in Beauregard leaves grown in the high tunnels versus the open field. Hence, our overall results indicate that growth stages and/or environment affect anthocyanin content of various sweet potato varieties, but that effect is highly variable and genotype specific.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bovell-Benjamin AC. Sweet potato: A review of its past, present and future role in human nutrition. Advances in Food and Nutrition Research. 2007;1–59.
2. Claessens L, Stoorvogel JJ, Antle JM. Ex ante assessment of dual-purpose sweet potato in the crop-livestock system of western Kenya: A minimum-data approach. Agric. Syst. 2008;99(1):13–22.
3. Islam S. Sweet potato (*Ipomoea batatas* L.) leaf: Its potential effect on human health and nutrition. J. Food Sci. 2006;71 (2):13–21.
4. Islam MS, Yoshimoto M, Yahara S, Okuno S, Ishiguro K, Yamakawa O. Identification and characterization of foliar polyphenolic composition in sweet potato (*Ipomoea batatas* L.) genotypes. J. Agric. Food Chem. 2002;50(13):3718–3722.
5. Yoshimoto M, Okuno S, Islam MS, Kurata RA, Yamakawa O. Polyphenolic content and antimutagenicity of sweet potato leaves in relation to commercial vegetables. In Acta Horticulturae. 2003; 628:677–685.
6. Parker P, Kram KE, Hall DTT. Peer coaching: An untapped resource for development. Organ. Dyn. 2014;43(2): 122–129.
7. Islam MS, Yoshimoto M, Terahara N, Yamakawa O. Anthocyanin compositions in sweet potato (*Ipomoea batatas* L.) leaves. Biosci. Biotechnol. Biochem. 2002; 66(11):2483–2486.
8. Su X, Griffin J, Xu J, Ouyang P, Zhao Z, Wang W. Identification and quantification of anthocyanins in purple-fleshed sweet potato leaves. Heliyon. 2019;5(6): e01964.
9. Islam M, Yoshimoto M, Yamakawa O. Distribution and physiological functions of caffeoylquinic acid derivatives in leaves of sweet potato genotypes. J. Food Sci. 2003;68(1):111–116.
10. Meyer AS, Heinonen M, Frankel EN. Antioxidant interactions of catechin, cyanidin, caffeic acid, quercetin and ellagic acid on human LDL oxidation. Food Chem. 1998;61(1–2):71–75.
11. Lim S, Xu J, Kim J, Chen TY, Su X, Standard J, Carey E, Griffin J, Herndon B, Katz B, et al. Role of anthocyanin-enriched purple-fleshed sweet potato p40 in colorectal cancer prevention. Mol. Nutr. Food Res. 2013;57(11):1908–1917.
12. Johnson M, Pace RD. Sweet potato leaves: Properties and synergistic interactions that promote health and prevent disease. Nutr. Rev. 2010;68(10): 604–615.
13. Xu J, Su X, Li Y, Sun X, Wang D, Wang, W. Response of bioactive phytochemicals in vegetables and fruits to environmental factors. Eur. J. Nutr. Food Safety. 2019;9 (3):233-247.
14. Shao HB, Chu LY, Lu ZH, Kang CM. Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. Int. J. Biol. Sci. 2008;4 (1):8–14.
15. Shao H bo, Chu L ye, Shao M an, Ja hleel CA, Hong-mei M. Higher plant antioxidants and redox signaling under environmental stresses. Comptes Rendus - Biologies. 2008;433–441.
16. Çirak C, Radušiene J, Çamas N. Pseudohypericin and hyperforin in two Turkish Hypericum species: Variation among plant parts and phenological stages. Biochem. Syst. Ecol. 2008;36(5-6): 377–382.
17. Omezzine F, Haouala R. Effect of *Trigonella foenum-graecum* L. development stages on some phytochemicals content and allelopathic potential. Sci. Hortic. (Amsterdam). 2013; 160:335–344.
18. Taylor JLS, Van Staden J. The effect of age, season and growth conditions on anti-inflammatory activity in *Eucomis autumnalis* (Mill.) Chitt. Plant extracts. Plant Growth Regul. 2001;34(1): 39–47.
19. Xu J, Su X, Lim S, Griffin J, Carey E, Katz B, Tomich J, Smith JS, Wang W. Characterisation and stability of anthocyanins in purple-fleshed sweet potato P40. Food Chem. 2015;186: 90–96.
20. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J. Enol Vitic. 1965;16:144–158.

21. Wang LS, Stoner GD. Anthocyanins and their role in cancer prevention. Cancer Lett. 2008;269(2):281–290.
22. Lee MJ, Park JS, Choi DS, Jung MY. Characterization and quantitation of anthocyanins in purple-fleshed sweet potatoes cultivated in Korea by HPLC-DAD and HPLC-ESI-QTOF-MS/MS. J. Agric. Food Chem. 2013;61(12):3148–3158.
23. Fang ZX, Bhandari B. Effect of spray drying and storage on the stability of bayberry polyphenols. Food Chem. 2011; 129:1139–1147.

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