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Short Communication

Anthocyanin indexes, quercetin, kaempferol and myricetin concentration in leaves and fruit of *Abutilon theophrasti* Medik. genetic resources

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Abstract

Anthocyanin indexes, quercetin, kaempferol and myricetin may provide industry with potential new medicines or nutraceuticals. Velvetleaf (*Abutilon theophrasti* Medik) leaves from 42 plant introductions (PI) were analyzed for anthocyanin indexes while both leaves and fruit were used for quercetin, kaempferol and myricetin concentration analysis by reverse-phase high performance liquid chromatography. Leaf anthocyanin indexes ranged from 6.15 to 11.25 among PI. Leaf quercetin and kaempferol concentrations ranged from 1.50 to 4.79 mg/g and 0.43 to 2.17 mg/g, respectively. Fruit quercetin, kaempferol and myricetin concentrations ranged from 0.061 to 0.266 mg/g, 0.054 to 0.734 mg/g, and 0 to 35.87 μg/g, respectively. Significant differences in leaf weight were also observed. Significant correlations were found between several traits. This information regarding anthocyanin indexes, quercetin, kaempferol and myricetin concentrations will be useful for velvetleaf cultivar development. Breeders and other scientists could use this germplasm that contains high concentrations of anthocyanins, quercetin, kaempferol and myricetin to develop new medicines or nutraceuticals from an extremely useful weedy species.

Keywords: *Abutilon theophrasti*; anthocyanin index; HPLC; kaempferol; myricetin; quercetin

Experiment

Velvetleaf (*Abutilon theophrasti* Medik) is a weed in corn, soybean and sorghum (Stegink and Spencer, 1988) in the United States. However, it has potential for use as a forage crop for sheep (Marten and Andersen, 1975) and has been used as an ethno-veterinary medicinal plant in Pakistan, where it is used for ephemeral fever (Khan and Hanif, 2006). Anthocyanins have shown chemopreventive effects (Thomasset *et al.*, 2009), and the flavonols, quercetin, kaempferol and myricetin have been clinically shown to have a preventive effect on pancreatic cancer (Nothlings *et al.*, 2007). Although flavonoids have been found in velvetleaf flowers (Matlawska and Sikorska, 2005), variation in anthocyanin and flavonol content in leaves and fruit is limited. Therefore, the objectives of this study were to (1) determine the concentration of anthocyanin indexes, quercetin, kaempferol and myricetin in velvetleaf PI in the USDA germplasm collection using reverse-phase high performance liquid

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chromatography (HPLC) and (2) determine correlations between anthocyanin indexes, quercetin, kaempferol and myricetin in velvetleaf.

Forty-two PI from the USDA velvetleaf germplasm collection were selected. Due to its high outcrossing, seeds from each accession were planted in 6-m long plots with at least two different species serving as borders in Griffin, Georgia during 2009. Leaves and fruit were collected from each accession after approximately 8 weeks of growth and stored at −20°C until analysis. An Opti Sciences CCM-200 chlorophyll content meter was converted to a hand-held anthocyanin meter. The manufacturer replaced the 655 nm light emitting diode (LED) of the CCM with a 520 nm LED to measure the absorbance near the wavelength at which free anthocyanin aglycones, cyanidin and pelargonidin monoglucosides absorb (Macz-Pop *et al*., 2004). Anthocyanin indexes were determined by inserting each leaf between the meter and the LED diode, followed by gently pressing the LED directly on to the leaf and recording from each of two leaves of velvetleaf PI growing in the field on 22 July 2009.

Velvetleaf and fruit tissues were ground to fine powder with liquid nitrogen. Approximately, 0.3 g of ground tissue was placed into tubes in duplicate, and weights were recorded. Extraction solvent (6 ml) consisting of 60% HPLC-grade methanol with 1.2 M HCl was added to each tube, and samples were mixed and incubated at 80°C for 2 h. The samples were then centrifuged, and a portion of the supernatant was filtered through a 0.45 μM membrane prior to injection. Separations were performed by reverse-phase HPLC using a Zorbax Eclipse 3.0 × 150 mm, 5 μm, C18 column (Agilent Technologies) at 40°C on an Agilent 1100 with a binary pump and autosampler. The sample injection volume was 5 μl, and analytes were monitored with a diode-array detector at 370 nm (flavonols). Flavonoid standards including quercetin (catalogue no. Q4951), kaempferol (catalogue no. 60 010) and myricetin (catalogue no. M6760) (Sigma-Aldrich, St. Louis, MO) were dissolved in a 5:3:2 mix of DMSO, methanol, water and diluted with 60% methanol to generate standard curves for peak identification and quantitation. Two mobile phases were used. Mobile phase 1 consisted of HPLC-grade acetonitrile (B) and 0.1% formic acid in filtered, sterile water (A). The flow rate was 0.8 ml/min at the following gradient: 15% B at time zero to 35% B at 20 min. Mobile phase 2 consisted of HPLC-grade methanol and HPLC-grade acetonitrile in a 2:1 mix (B) and 0.1% formic acid in (A). The flow rate was 0.8 ml/min at the following gradient: 15% B at time zero to 52.5% B at 25 min. The column was washed with 95% B for 5 min and equilibrated for 7 min between injections. Mobile phase 1 resolved kaempferol (flavonol), and quercetin (flavonol) was co-eluted. Mobile phase 2 resolved quercetin, while kaempferol was co-eluted. The range of concentration for the linear calibration curve was 1–400 ng/μl for quercetin and kaempferol; however, the calibration curve was 1–20 ng/μl for myricetin. Duplicate extractions and injections in each mobile phase ensured adequate separation and quantification of all flavonols in each sample. Two extractions per sample accession were used for data analysis. One-way analysis of variance was conducted using the statistical analysis system (SAS, 2008) to analyze the data and Duncan's multiple range test was used to separate means. Pearson's correlation coefficient analysis was performed to determine relationships between the flavonols and anthocyanin indexes.

Discussion

Anthocyanin indexes, quercetin and kaempferol concen trations from leaf tissue are summarized in Supplementary Table S1, available online only at http://journals.cambridge.org while quercetin, kaempferol and myricetin concentrations from fruit tissue are summarized in Supplementary Table S2, available online only at http://journals.cambridge.org. Among the 42 PI quantified, the leaves of PI 499257 contained the significantly highest anthocyanin index (11.25). The lowest leaf anthocyanin index producing samples included the French and Russian Federation PI, PI 499233 (6.95) and PI 499240 (6.15), respectively, and were significantly lower than four velvetleaf PI. Both PI 499246 and PI 499213 from Japan produced significantly more quercetin (4.79 and 4.56 mg/g, respectively) concentration than all other PI and PI 499253 from the former Soviet Union produced the lowest concentration of quercetin (1.50 mg/g). PI 499233 and PI 499246 produced significantly more kaempferol (2.17 and 1.97 mg/g, respectively) concentration than all other PI while the lowest concentration was observed in PI 499225 from Switzerland. Only 26 PI were evaluated for quercetin, kaempferol and myricetin concentration in their fruit because the other 16 velvetleaf PI fruit were damaged by the scentless plant bug (*Niesthrea louisianica*). Anthocyanin indexes were not recorded for velvetleaf fruit because the LED diode was too small to adequately measure the larger fruit capsules. Significant differences occurred for quercetin, kaempferol and myricetin concentraiton in the fruit of these 26 PI. PI 499249 (Poland), PI 499222 (Portugal), PI 499223 (India) and PI 499253 produced significantly higher quercetin concentration ranging from 0.242 to 0.299 mg/g while, PI 499256 from the Russian Federation produced the lowest quercetin (0.061 mg/g) concentration in their fruit. Both PI 499253 and PI 499216 produced significantly

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higher amount of kaempferol (0.734 and 0.565 mg/g, respectively) concentration and PI 499236 produced the lowest amount of kaempferol (0.054 mg/g) concentration. PI 499222, 499227 (Russian Federation), 499232 (Sweden), 499238 (Ukraine), 499225, 499249, 499236, 499223 and 499239 (The Netherlands) produced significantly higher amounts of myricetin concentration in their fruit ranging from 10.11 to 35.87 μg/g. All other PI did not produce any measurable myricetin.

The weights for leaves and fruits are listed in Supplementary Tables S1 and S2, available online only at http://journals.cambridge.org respectively. Significant variation in leaf weights were observed at $P < 0.0001$ but not in fruit weight. Leaf anthocyanin index significantly correlated with quercetin ($r^2 = -0.42^{***}$, $n = 42$) and kaempferol ($r^2 = -0.52^{***}$) concentration, while quercetin significantly correlated with kaempferol ($r^2 = 0.74^{***}$, $n = 42$) concentration. In addition, fruit quercetin significantly correlated with myricetin ($r^2 = 0.64^{***}$, $n = 26$) concentration, and fruit kaempferol significantly correlated with myricetin ($r^2 = -0.57^{***}$, $n = 26$) concentration.

Significant variability was observed in the anthocyanin indexes, concentrations of quercetin, kaempferol and myricetin among 42 velvetleaf PI leaves and 26 velvetleaf PI fruit. Anthocyanin estimations were consistent because they were applied to similar environmental conditions at Griffin, GA and provide a relative anthocyanin content value in velvetleaf leaves and fruit. This study showed that velvetleaf leaves contain higher amounts of quercetin (averaging 3.06 mg/g) than red raw cabbage (0.004 mg/g).

Kaempferol produced from velvetleaf leaves averaged 1.63 mg/g while red raw cabbage does not produce it. Velvetleaf fruit averaged 0.15 mg/g of quercetin while raw squash produces about 0.007 mg/g of quercetin. These traits could be added to a breeding programme to develop velvetleaf cultivars with improved anthocyanin index, quercetin, kaempferol and myricetin content. Velvetleaf could provide humans and animals with nutraceutical, medicinal, or functional food products, since these leaves and fruit contain high amounts of quercetin that could complement their use as a functional forage species.

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