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Short communication

Anthocyanin and chlorophyll content during poinsettia bract development

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ABSTRACT

The concentration of anthocyanins, chlorophyll a and b were quantified during bract development of three differently colored *Euphorbia pulcherrima* Willd. cultivars ('Mars White', 'Mars Pink', and 'Mira Red') and bract color was colorimetrically determined. Color parameter *a** increased from the first analyzed stage of bract development to the subsequent stages in all poinsettia cultivars. Chlorophyll a predominated over chlorophyll b in partially pigmented bracts and a sharp decrease in chlorophyll a content was detected in fully pigmented bracts. Eleven different anthocyanic pigments were identified with the use of high-performance liquid chromatography/mass spectrometry (HPLC/MS). The predominant cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-glucoside and pelargonidin-3-rutinoside were also detected in white and pink poinsettia bracts. Anthocyanin content levels increased significantly with the transition from partially to fully pigmented bracts in all analyzed cultivars and their accumulation coincided with the arrest of photosynthetic pigments synthesis.

1. Introduction

Poinsettia (*Euphorbia pulcherrima* Willd.) is a worldwide popular potted plant and a traditional ornamental, valued for its red colored leaves during the Christmas season. During the past years new cultivars are available in the market mainly differentiated by their height, size of the leaves and intense colored bracts.

A broad spectrum of plant tissue colors can be attributed to the presence of pigments in the vacuoles of the epidermal cells (Eugster and Markifischer, 1991). Green leaves and red bracts of poinsettia accumulate different groups of pigments; in the latter, anthocyanins predominate over chlorophylls (Pomar and Barceló, 2007). Several cyanidin and pelargonidin based anthocyanins with different sugar moieties attached have been identified so far (Asen, 1958, 1979) directly influencing the color and marketability of poinsettia (Bennett et al., 2008). In addition to genetically controlled pigment levels, their content is dependent on the developmental stage. In roses, with the progression of flower development, changes in anthocyanin content have been reported (Kumar et al., 2008; Schmitzer et al., 2009a, 2010). It seems that anthocyanin biosynthesis in poinsettia bracts is similarly regulated.

Potted poinsettias have become increasingly popular over the past decades. In the present investigation we aimed to identify several novel individual anthocyanins in differently colored poinsettia cultivars with the use of MS and thus enhance the natural product database. Particularly, pink and white poinsettias were biochemically studied for the first time. The changes in the content of specific and total pigments during bract development were also evaluated. To our knowledge this work provides the first report on the abundance of specific pigments at different stages of poinsettia bract development and represents a valuable insight into their gradual coloration.

2. Materials and methods

2.1. Plant material

Rooted cuttings of three poinsettia cultivars, 'Mars Pink', 'Mars White', and 'Mira Red', were potted in 12 cm pots containing Plantaflor® substrate (pH 5.2–6.0; 130–300 mg L^{-1} N, 70–170 mg L^{-1} P_2O_5, 140–300 mg L^{-1} K_2O) on 25 July 2011 and grown in a controlled environment glass greenhouse at 22/18 °C (day/night) under natural photoperiod and 75–85% relative humidity. Flood irrigation system with 4 min water (18 °C) supply was used daily. Plants were pinched on 15 August leaving approx. 6 nodes and on 5 December three bracts from each plant (15 per cultivar) were analyzed at four developmental stages: (1), partially colored bracts; (2), fully pigmented bracts; (3); bracts start to show a decrease in coloration; and (4), bract discoloration apparent. The cultivars were chosen for their differently colored bracts (white, pink and red), each representing one of the top cultivars according to consumer preferences, to best evaluate the variety and content levels of different anthocyanins in poinsettia.

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2.2. Bract color measurements

Bract color was measured with a portable colorimeter (CR-10 Chroma; Minolta, Osaka, Japan) calibrated with a white standard calibration plate before use. The L^* value ranges from 0 to 100 (0 = black, 100 = white), and a^* and b^* values extend from −60 (green and blue) to 60 (red and yellow). Color was measured in the middle of each bract near the main vein (four replicates per bract) to ensure equal measurement conditions.

2.3. Pigment identification

The content of chlorophyll a and b was evaluated according to the method by Wellburn (1994). 1.5 mL microtubes were filled with 0.5 mL dimethyl sulphoxide (DMSO) and one 4 mm leaf disc together with crystals of magnesium hydroxide carbonate were added and mashed for better extraction. 0.5 mL of DMSO solution was added and samples were left in a water bath (65 °C) in the dark for 2 h. Samples were cooled to room temperature, decanted into vessels and absorption was measured on a Lambda Bio spectrometer (Perkin Elmer, Waltham, MA) at 649 nm (chlorophyll b) and 665 nm (chlorophyll a). Concentrations of photosynthetic pigments in extracts were determined by Wellburn (1994).

For anthocyanin quantification bracts were ground with liquid nitrogen and 0.5 g of powder was extracted with 2 mL MeOH containing 3% (v/v) HCOOH and 1% (w/v) BHT in an ultrasonic bath for 1 h. Samples were centrifuged for 7 min at 12,000 × *g*, filtered through a Chromafil AO-45/25 polyamide filter (Macherey-Nagel, Düren, Germany) and analyzed using a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA) with a diode array detector at 530 nm. A Phenomenex (Torrance, CA) HPLC column C18 (150 mm × 4.6 mm, Gemini 3 μ) protected with a Phenomenex security column operated at 25 °C was used. The injection volume was 20 μL, flow rate was 1 mL min^{-1}. The elution solvents were aqueous 1% formic acid and 5% acetonitrile (A) and 100% acetonitrile (B). Samples were eluted according to the linear gradient described by Marks et al. (2007). Anthocyanins were identified using a mass spectrometer (LCQ Deca XP MAX, Thermo Scientific) with electrospray ionization (ESI) operating in positive ion mode using MS^2 scanning mode from m/z 115 to 800. The injection volume was 10 μL and the flow rate 1 mL min^{-1}. Anthocyanins were quantified with the use of external standards or similar compounds (Table 1) and expressed in mg kg^{-1} FW.

2.4. Statistical analysis

The results were analyzed with Statgraphics Plus 4.0 (Manugistics, Rockville, MD) program using one-way analysis of variance (ANOVA) and Duncan's multiple range test ($P < 0.05$). Differences in measured parameters among cultivars were analyzed in fully pigmented bracts.

3. Results and discussion

3.1. Bract colorimetric parameters

Parameter a^* is associated with red coloration of different plant organs (Schmitzer et al., 2009a,b, 2010) and closely related to visual horticultural attributes of ornamentals. Color parameter a^* increased from the first analyzed stage of bract development to the subsequent stages in all poinsettia cultivars (Table 2). Interestingly, an increase of parameter a^* was detected in white bracts, suggesting that pale rosy hues are distinctive for these cultivars. Parameter b^* decreased in 'Mars Pink' and 'Mars White' poinsettia cultivars and increased in 'Mira Red' cultivar. With the progression of bract development a increase of parameter L^* was observed in white and red cultivars and a decrease in salmon pink cultivar. Bract development is as such not only characterized by their expansion and morphogenesis (Ayala Arreola et al., 2008) but can directly be described with altered colorimetric parameters.

3.2. Pigment accumulation in poinsettia bracts

Chlorophyll a predominated over chlorophyll b in partially colored bracts, a sharp decrease in its content was measured in fully pigmented bracts, followed by a moderate increase at later stages (Table 2). It seems that photosynthetic pigments are thus synthesized in the first stages of bract development and are later replaced by different phenolic compounds. Similarly, Ayala Arreola et al. (2008) measured a significant decrease of individual/total chlorophylls from the beginning of bract pigmentation. Kannangara and Hansson (1998) determined that the arrest of chlorophyll formation could be attributed to the loss of protein content and enzymes in the chlorophyll biosynthesis pathway.

The HPLC chromatogram of bract extracts measured at 530 nm revealed 11 different peaks (Table 1). Cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-glucoside and pelargonidin-3-rutinoside were identified in white, salmon pink and red bracts in highest concentrations (Table 3). Minor cyanidin glycosides (cyanidin-3-rhamnoside, cyanidin-3-xyloside, cyanidin-3-(6″-acetylglucoside)-5-glucoside and cyanidin-3-(6″-malonylglucoside)-5-glucoside), pelargonidin-3-(6″-malonylglucoside) and delphinidin-3-(2G-xylosylrutinoside) were only detected in pink and red bracts in lower concentrations (data not presented). Interestingly, white cultivars contained a few prevailing anthocyanins also detected in the bracts of the originally red *E. pulcherrima* species. The same applies to white roses, which accumulate several cyanidin and pelargonidin based

Table 1
A list of anthocyanins identified in three poinsettia cultivars, standards used for calculations and MS specifications.

Anthocyanin[a]	Standard used	Poinsettia cultivar			$[M^+]$ (m/z)	MS^2 $[M^+]$ (m/z)
		'Mars Pink'	'Mars White'	'Mira Red'	*n*	
cy-3-Galactoside	cy-3-Galactoside	×	×	×	449	287
cy-3-Glucoside	cy-3-Glucoside	×	×	×	449	287
cy-3-Rutinoside	cy-3-Rutinoside	×	×	×	595	449/287
pel-3-Glucoside	cy-3-Glucoside	×	×	×	433	271
pel-3-Rutinoside	cy-3-Rutinoside	×	×	×	579	433/271
cy-3-Rhamnoside	cy-3-Glucoside			×	433	287
cy-3-Xyloside	cy-3-Glucoside	×		×	419	287
cy-3-(6"Acetylglucoside)-5-glucoside	cy-3-Glucoside	×		×	653	287
cy-3-(6"Malonylglucoside)-5-glucoside	cy-3-Glucoside	×		×	697	535/449/287
pel-3-(6"Malonylglucoside)	cy-3-Glucoside	×		×	519	433/271
del-3-(2G-Xylosylrutinoside)	cy-3-Glucoside			×	743	597/435/303

[a] Anthocyanin: cy, cyanidin; pel, pelargonidin; del, delphinidin.