UNIVERZA V LJUBLJANI
BIOTEHNIŠKA FAKULTETA

Valentina SCHMITZER

POVEZAVE MED DEJAVNIKI, KI VPLIVAJO NA BARVO, IN FENOLNIMI SNOVMI PRI IZBRANIH OKRASNIH RASTLINAH

DOKTORSKA DISERTACIJA

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CORRELATION BETWEEN COLOR INFLUENCING FACTORS AND PHENOLICS IN SELECTED ORNAMENTAL PLANTS

DOCTORAL DISSERTATION

Ljubljana, 2012

Na podlagi Statuta Univerze v Ljubljani ter po sklepu Senata Biotehniške fakultete in sklepa senata Univerze v Ljubljani z dne 28. 9. 2009 je bilo potrjeno, da kandidatka izpolnjuje pogoje za neposreden prehod na Podiplomski študij bioloških in biotehniških znanosti ter opravljanje doktorata znanosti s področja agronomije. Za mentorja je bil imenovan prof. dr. Franci ŠTAMPAR.

Praktičen del poskusov je bil izveden v rastlinjakih Biotehniške fakultete, Oddelka za agronomijo, vzorčenja za del poskusov pa so bila opravljena v Arboretumu Volčji potok in Botaničnem vrtu Univerze v Ljubljani. Laboratorijski del poskusov je bil izveden na Katedri za sadjarstvo, vinogradništvo in vrtnarstvo Oddelka za agronomijo Biotehniške fakultete v Ljubljani.

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Datum zagovora: 23.3.2012

Naloga je rezultat lastnega raziskovalnega dela. Izjavljam, da so vsa vključena znanstvena dela identična objavljeni verziji.

Valentina SCHMITZER

KLJUČNA DOKUMENTACIJSKA INFORMACIJA

ŠD Dd
DK UDK 635.92:582.734:581.14:547.973(043.3)
KG okrasne rastline/vrtnice/javorji/barva/antociani
KK AGRIS F0/F62
AV SCHMITZER, Valentina, univ. dipl. inž. krajinske arhitekture, dipl. inž. agronomije
SA ŠTAMPAR, Franci (mentor)
KZ SI-1000 Ljubljana, Jamnikarjeva 101
ZA Univerza v Ljubljani, Biotehniška fakulteta, Podiplomski študij bioloških in biotehniških znanosti, področje agronomije
LI 2012
IN POVEZAVE MED DEJAVNIKI, KI VPLIVAJO NA BARVO, IN FENOLNIMI SNOVMI PRI IZBRANIH OKRASNIH RASTLINAH
TD Doktorska disertacija
OP VI, 64 str., 3 sl., 53 vir.
IJ sl
JI sl/en
AL Doktorsko delo obravnava doslej malo raziskane povezave med notranjimi dejavniki, ki vplivajo na barvo okrasnih rastlin, in vsebnostjo pigmentov v listih in cvetovih. Postopna jesenska rdeča obarvanost listov pahljačastih javorjev je bila sortno in pozicijsko pogojena. Količina rdeče obarvanosti in vsebnosti prevladujočih antocianinov cianidin-3-glukozida in cianidin-3-rutinozida so bile največje v listih z vrha krošnje in najmanjše v listih z vej, ki so izraščale s spodnjega dela provodnika. Korelacijske analize so potrdile povezanost barvnih parametrov z vsebnostjo prevladujočega antocianina v listih vseh analiziranih sort. Pri sorti miniaturne vrtnice in osmih sortah pokrovnih vrtnic smo ob spremljanju razvoja cveta ugotovili povečanje svetlosti venčnih listov, pri rdeče in rožnato obarvanih sortah pa zmanjšanje rdeče obarvanosti od faze popka do senescentnega cveta. Statistično značilno je bil na isti rastlini najtemnejši popek, delno odprt in popolnoma odprt cvet svetlejša, senescentni cvetovi najsvetlejši. Pri obarvanih sortah smo v fazi senescentnega cveta izmerili prehod v modrikaste tone, ki je v korelaciji s povečanjem pH vrednostjo celičnega soka. V venčnih listih rdečih in rožnatih vrtnic sta prevladovala antocianina pelargonidin-3,5-di-glukozid in cianidin-3,5-di-glukozid. Vsebnost prevladujočih in skupnih antocianinov je naraščala od popka do popolnoma odprtega cveta in se zmanjšala v senescentnih cvetovih. Med barvnim parametrom *a** in skupnimi antocianini so bile pri vseh sortah izmerjene tesne korelacije. Z razvojem cveta se je vsebnost prevladujočih kvercetin-3-ramnozida in kvercetin-3-rutinozida močno zmanjšala. Podobno zmanjšanje smo zaznali tudi v vsebnosti katehina ter kavine, klorogenske, galne, protokatehulne in *p*-kumarne kisline. Pri miniaturni vrtnici, posajeni v izrazito kisel (pH vrednost 3,3) in rahlo bazičen (pH vrednost 7,3) substrat, smo opazili zmanjšano tvorbo cvetov in večjo vsebnost prevladujočih in skupnih antocianinov, kvercetinov, katehina in fenolnih kislin v primerjavi z rastlinami, posajenimi v substratno mešanico rahlo kisle pH vrednosti (pH 4,7).

KEY WORDS DOCUMENTATION

DN Dd
DC UDC 635.92:582.734:581.14:547.973(043.3)
CX ornamental plants/roses/maples/color/anthocyanins
CC AGRIS F01/F62
AU SCHMITZER, Valentina
AA ŠTAMPAR, Franci (supervisor)
PP SI- 1000, Ljubljana, Jamnikarjeva 101
PB University of Ljubljana, Biotechnical Faculty, Postgraduate study of Biological and Biotechnical Sciences, Field: Agronomy
PY 2012
TI CORRELATION BETWEEN COLOR INFLUENCING FACTORS AND PHENOLICS IN SELECTED ORNAMENTAL PLANTS
DT Doctoral Dissertation
NO VI, 64 p., 3 fig., 53 ref.
LA sl
AL sl/en
AB The thesis elucidated correlations between several internal factors contributing to tissue color and links them to the content of pigments in leaves and petals. The gradual autumn coloration is cultivar and position dependent as the amount of red coloration and the content of major anthocyanins cyanidin-3-glucoside and cyanidin-3-rutinoside was always highest in leaves collected from branches from the top of the canopy and lowest in leaves of the base branches. Correlation analysis confirmed the relationship of color parameters with the predominant anthocyanin content in leaves of all cultivars analyzed. During rose flower development an increase of petal lightness was observed in the analyzed miniature rose cultivar of and eight cultivars of groundcover roses. In red and pink cultivars, a decrease of red coloration was confirmed from the bud to the senescent flower stage. On the same plant, buds were the darkest, partially opened and fully opened flowers were lighter and senescent flowers were statistically the most pale. A transition to bluish hues was detected in senescent flowers of red and pink cultivars and correlated with an increased cell sap pH level. Pelargonidin-3,5-di-glucoside and cyanidin-3,5-di-glucoside were the predominant anthocyanins in rose petals. The content of the predominant and total anthocyanins increased from the bud stage to the totally opened flower stage and decreased in senescent flowers. Tight correlations were determined between the color parameter *a** and total anthocyanins in all cultivars analyzed. The content of the predominant quercetins-3-rhamnoside and quercetins-3-rutinoside decreased with flower development and a similar trend was observed in the content of catechin, caffeic, chlorogenic, gallic, protocatechulic and *p*-coumaric acid. In miniature roses, potted in highly acidic (pH 3.3) and slightly alkaline (pH 7.3) substrate a decrease in the formation of flowers and an increase in the content of predominant and total anthocyanins as well as quercetins, catechin and phenolic acids was detected in comparison to slightly acidic (pH 4.7) substrate.

KAZALO VSEBINE

KAZALO SLIK

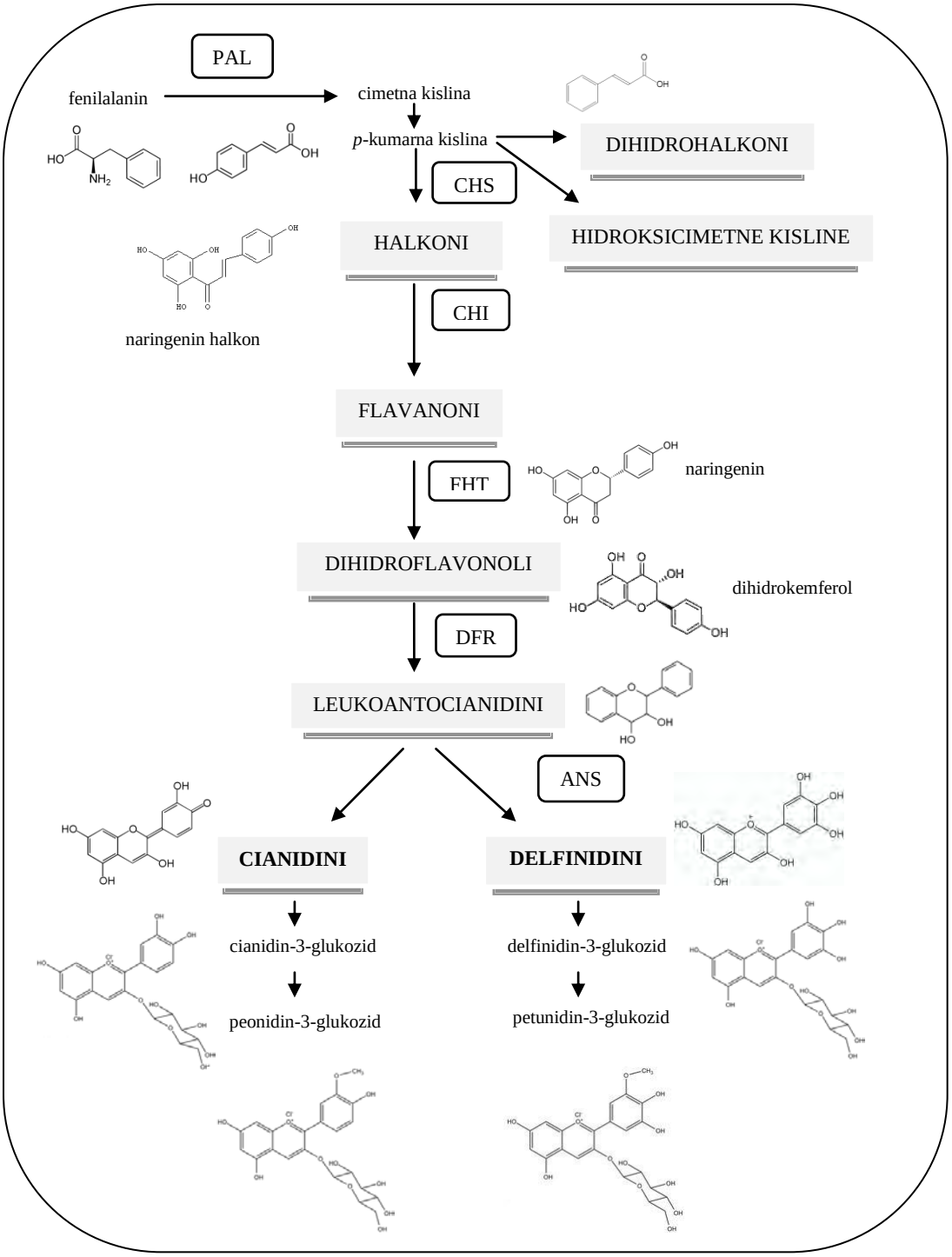
1 UVOD

Okrasne rastline predstavljajo široko skupino rastlin, katerih osnovni namen je popestritev bivalnega in kulturnega okolja. Njihovo sajenje je namenjeno estetski funkciji, ki prevladuje nad uporabnimi, ekološkimi, sociološkimi in drugimi pomeni. Največkrat so okrasne rastline cenjene zaradi svojih cvetov, listov, plodov, lubja, oblike rasti in drugih posebnosti (prijeten vonj, zanimivi trni itd.). Med najpomembnejšimi vizualnimi lastnostmi okrasnih rastlin posebej izstopa barva različnih tkiv in organov. Širok barvni spekter rastlinskih tkiv je v veliki meri posledica vsebnosti različnih pigmentov (karotenoidov, betalainov, antocianov in drugih flavonoidov kot so flavoni in flavonoli) v vakuolah epidermalnih in perifernih mezofilnih celic (Eugster in Markifischer, 1991; Cooper-Driver, 2001).

Med rdečimi pigmenti so v vakuolah najpogosteje zastopani antocianini, vodotopni rastlinski pigmenti, ki imajo na benzenov obroč aglikona vezano sladkorno enoto, običajno glukozo, galaktozo, ramnozo, arabinozo, ksilozo ali kak drug sladkor (Mikanagi in sod., 1995). Sladkorji so na aglikone praviloma vezani na tretjem (ali redkeje petem) ogljikovem atomu benzenovega obroča, le izjemoma na drugih pozicijah (Cooper-Driver, 2001). Glede na število vezanih sladkornih enot ločimo mono-, di- ali tri-glikozide (Ougham in sod., 2005).

V dosedanjih raziskavah so v tkivih okrasnih rastlin potrdili prisotnost glikozidov cianidina, delfinidina, pelargonidina, malvidina, petunidina in peonidina ter redkejših aurantinidina, europinidina, luteolinidina in rosinidina (Toki in sod., 2008; Guzmán in sod., 2009). Najpogostejši kromofori so pelargonidin, cianidin in delfinidin; v vakuolah rdečih in vijolično-rdečkastih cvetov so v večjih koncentracijah zastopani cianidini, v rožnatih in oranžnih petalih pelargonidini in v modrikastih cvetnih listih delfinidini (Cooper-Driver, 2001). Antocianini so prisotni tudi v vegetativnih tkivih (listih, steblih, koreninah, mladih poganjkih) in so zlasti opazni v jesenskem času, ko se listje obarva, in v spomladanskih mladih poganjkih in listih (Lee, 2002).

Antociane uvrščamo v raznoliko skupino fenolnih snovi, natančneje flavonoidov, ki nastajajo v sintezni poti sekundarnega metabolizma. Iz fenilalanina se ob katalizi encima fenilalanin amonijak liaze (PAL) tvori cimetna kislina, ki se s hidroksilacijo pretvori v *p*-kumarno kislino. Ta je prekurzor za hidroksicimetne kisline (kavno, klorogensko in ferulno kislino), dihidrihalkone in halkone. Med slednjimi je za sintezo antocianov pomemben naringenin halkon iz katerega se ob sodelovanju encima halkon sintaze (CHS) tvori naringenin in kasneje dihidroflavonoli ter leukoantocianidini. Ti so prekurzorji za cianidine, peonidine, delfinidine in petunidine, ki pomembno prispevajo k obarvanosti rastlinskih tkiv (Cooper-Driver, 2001).



Slika 1: Biosistemska pot antocianinov in drugih fenolnih snovi. PAL, fenilalanin amonijak liaza; CHS, halkon sintaza; CHI, halkon izomeraza; FHT, flavanon-3-hidroksilaza; DFR, dihidroflavonol-4-reduktaza; ANS, antocianin sintaza (prirejeno po Cooper-Driver, 2001)
Figure 1: Biosystemic pathway of anthocyanins and selected phenolics. PAL, phenylalanin ammonia lyase; CHS, chalcone synthase; CHI, chalcone isomerase; FHT, flavanone-3-hydroxylase; DFR, dihydroflavonol-4-reductase; ANS, anthocyanin synthase (adapted after Cooper-Driver, 2001)

Antociani poleg pigmentacije generativnega rastlinskega tkiva (z namenom privabljanja opraševalcev in raznašalcev semen) prispevajo k obrambi pred različnimi oblikami stresa (Gould in sod., 1995; Cooper-Driver, 2001). Največkrat so prisotnih v mladih listih in poganjkih, vegetativnih tkivih tropskih rastlin, starajočih (senescentnih) listih in rastlinskih tkivih, ki so bila podvržena različnim stresnim dejavnikom. Gould in sod. (1995) opisujejo vlogo antocianov v procesu fotoinhibicije, saj absorbirajo valovne dolžine med 520 in 530 nm, podobno kot klorofil b. Z absorbcijo presežne svetlobe zmanjšajo možnost poškodb in hkrati povečajo fotosintetsko aktivnost zelenih delov rastlin. Obenem antociani v vakuolah reagirajo s prostimi radikali in kovinskimi ioni ter delujejo kot antioksidanti.

Poleg glavnih pigmentov pri izražanju barve sodelujejo tudi kopigmenti iz skupine fenolnih snovi (kvercetini, klorogenska kislina, ferulna kislina itd.), ki z antocianinskimi pigmenti tvorijo komplekse (Davies in Mazza, 1993; Figueiredo in sod., 1996), največkrat preko sladkorne enote. Kompleksi so še posebej pomembni v rahlo kislih medijih (pH celičnega soka se giblje med 4 in 6), v katerih so antocianini večinoma prisotni v stabilni brezbarvni obliki (Cooper-Driver, 2001). Antocianini absorbirajo svetlobo valovne dolžine med 270 in 290 nm in vidni del spektra med 500 in 600 nm, preostali del pa odbijejo, kar se vizualno kaže v modrih, vijoličastih in rdečih barvnih odtenkih. Na absorpcijo svetlobe pa vplivata tako struktura antocianina kot tudi pH vrednost okolja (Gould, 2004).

Fenolne snovi v tkivih biokemijsko določamo s pomočjo tekočinske visokoločljivostne kromatografije. Ta temelji na primerjavi UV-Vis spektrov ter retencijskih časov posameznih komponent analita z znanimi standardnimi spojinami in na ta način omogoča njihovo identifikacijo in kvantifikacijo. Za natančnejšo določitev in potrditev posameznih fenolnih snovi v vzorcu so v uporabi masni spektrometri, ki ločijo ione glede na njihovo maso in naboj. Masna spektrometrija je ena najbolj občutljivih mikroanaliznih metod za analizo organskih in anorganskih spojin ter elementov v vzorcih. Pigmenti v celicah neposredno vplivajo na barvo rastlinskih tkiv, ki jo lahko opišemo vizualno, v strokovni literaturi pa zasledimo različne metodologije za določanje barve, ki so veliko bolj natančne (Lancaster in sod., 1997; Gonnet, 1998). Najpogosteje so v rabi kolorimetrični pripomočki (prenosni kolorimetri) za določanje odtenkov, nasičenosti in svetlosti barve, ki opišejo podatke s pomočjo CIE LAB parametrov (Lancaster in sod., 1997). Barvna koordinata *a**, ki je podana v merski lestvici od -60 do +60, s svojo višjo vrednostjo do določene mere predstavlja količino antocianskih (»rdečih«) pigmentov v tkivu. Vsebnost rastlinskih pigmentov in kolorimetrični parametri so pogosto v tesni zvezi in avtorji poročajo o njihovi korelaciji (Jia in sod., 2008). Do sedaj so bile raziskave o povezavi med antociani in izmerjenimi barvnimi parametri opravljene predvsem na plodovih sadnih rastlin (Iglesias in sod., 2008) in zelenjadnic (Lancaster in sod., 1997), manj pa je podatkov o korelacijah na okrasnih rastlinah (Katori in sod., 2002).

Pahljačasti javorji in njihova intenzivna barva listja predstavljajo odličen študijski model za opazovanje in analizo prehoda rdeče jesenske obarvanosti in spremljanja vsebnosti antocianinov v listih. Povezave med izmerjeno barvo in količino prevladujočih antocianinov v listih so bile predmet prvega sklopa poskusov doktorskega dela.

PAHLJAČASTI JAVORJI

Pahljačasti javorji (*Acer palmatum* Thunb.) so zaradi izjemne pestrosti barvnih odtenkov listja, ki je bodisi rdečkasto obarvano celo leto ali pa intenzivneje pordeči v jesenskem času, priljubljene okrasne rastline v vrtovih in javnih zasaditvah (Ji in sod., 1992). Rdeča barva listov javorjev je v veliki meri pogojena z vsebnostjo antocianinov v vakuolah celic epiderma in gobastega mezofila (Hughes in sod., 2007) in je odvisna od genotipa ter številnih okoljskih dejavnikov kot so temperatura, vsebnost hranil v tleh, osvetlitev in prisotnost patogenov ter različnih mehanskih dejavnikov, ki povzročajo stres (Davies, 2004).

Cianidin-3-glukozid je najpogosteje zastopan cianidin v listih kritosemenk (Harborne, 1976; Ishikura, 1976), raziskave pa so pokazale, da ta antocianin prevladuje tudi v listih požlahtnjenih rdečih okrasnih sort okrasnih lesnatih rastlin (Hughes in sod., 2007). V manjši meri so v listih kritosemenk prisotni tudi cianidin-3-rutinozid in drugi derivati cianidina ter delfinidina (Fossen in Anderssen, 1999). Analize listnega tkiva več sort pahljačastih javorjev, opravljene s pomočjo visokoločljivostne tekočinske kromatografije (high performance liquid chromatography – HPLC), so potrdile prisotnost dveh glavnih antocianinov, cianidin-3-glukozida in cianidin-3-rutinozida, ter številnih drugih antocianinov (predvsem glukozidov cianidina in delfinidina) v posameznih sortah v manjših koncentracijah (Ji in sod., 1992; Fossen in Andersen, 1999). Kombinacije pigmentov in drugi dejavniki jeseni vplivajo na obarvanje listov pahljačastih javorjev od intenzivne oranžne do temno vijoličaste barve.

Podobno, kot pri številnih drevesnih vrstah, se listi pahljačastih javorjev jeseni postopno obarvajo, kar je pogojeno s številnimi dejavniki - tudi s položajem veje na drevesu (topofiza). Fenolni in hormonski status, predvsem akumulacija antocianov ob istočasni razgradnji klorofila, se pri drevnini razlikuje glede na različno fiziološko starost posameznih delov rastline (Rey in sod., 1994). Topofiza opisuje fiziološko staranje delov lesnatih rastlin, ki se spreminja od debla (juvenilno) proti zunanjosti krošnje (senescentno). Poznavanje procesa topofize in določitev biokemijskih parametrov je bistveno predvsem za drevesničarsko prakso, saj je juvenilen material primernejši za koreninjenje in gojenje mladih rastlin (Osterc in sod., 2008). Biokemijski parametri (polifenoli, hormoni), s katerimi bi lahko razložili fenomen topofize, so do sedaj zelo malo raziskani. Markerji juvenilnosti pri lesnatih rastlinah tako temeljijo predvsem na morfoloških, fizioloških in histoloških spremembah (McGowran in sod., 1998; Nicolini in Chanson, 1999), biokemični parametri kot so encimi in fenoli (antociani) pa predstavljajo novost v raziskavah faznih sprememb in staranja (Valdés in sod., 2004), ki lahko nadgradijo dosedanje znanje o procesu staranja lesnatih rastlin.

V poskus spremljanja jesenskega obarvanja listov pri pahljačastih javorjih smo vključili sedem sort: osnovno vrsto *Acer palmatum* Thunb., *A. p.* var *palmatum*, *A. p.* 'Semi K2', *A. p.* 'Dissectum rubrifolium', *A. p.* 'Dissectum nigrum', *A. p.* 'Coreanum' in *A. p.* 'Bloodgood'. Vzorčili smo drevesa na treh lokacijah v Osrednji Sloveniji (Arboretum Volčji Potok,

Botanični vrt Univerze v Ljubljani in drevesnici Žiher-Špur pri Medvodah) sredi oktobra 2007. Drevesa so bila odrasla, v dobrem zdravstvenem stanju, sajena na polsenčni legi in z značilno jesensko obarvanimi listi. Na vsakem drevesu smo z vertikalnim pozicioniranjem določili tri obravnavanja (od podlage do vrha krošnje), glede na fiziološki status delov rastline:

(1) Terminalna veja (veja, ki izrašča iz vrha provodnika);
(2) Sredinska veja (veja, ki izrašča iz sredine provodnika);
(3) Bazalna veja (veja, ki izrašča iz dna provodnika).

Da bi omejili vpliv osvetlitve na vzorčenje smo na vsaki vzorčni veji vzorčili 5 listov le na koncu vej (poenoteno horizontalno pozicioniranje). Barvo smo izmerili v sredini vsakega lista s prenosnim kolorimetrom v treh ponovitvah in izvedli kemične analize vsebnosti izbranih sekundarnih metabolitov v vsakem listu posebej.

Postavili smo naslednje raziskovalne hipoteze:

- Jesenska obarvanost pri sortah pahljačastega javorja je odvisna od položaja veje na drevesu;
- Listi na terminalnih vejah so bolj obarvani in vsebujejo več antocianinov v primerjavi z listi na bazalnih vejah v isti časovni enoti;
- Vsebnost antocianinov v listih pahljačastih javorjev je sortno pogojena;
- Izmerjeni barvni parametri so v tesni korelaciji z vsebnostjo izbranih sekundarnih metabolitov.

VRTNICE

Med okrasnimi rastlinami vrtnice še vedno zasedajo prvo mesto predvsem pri gojenju za rezano cvetje, izjemno pa so priljubljene tudi kot rastline za zunanje zasaditve v parkih in zasebnih vrtovih. V zadnjih desetletjih miniaturne vrtnice pridobivajo tržni delež med lončnicami, predvsem zaradi intenzivnega cvetenja, hitre tvorbe novih cvetov, prikladne velikosti in cenovne dostopnosti. Številne sorte vrtnic odlikujejo različna oblika cvetov, velikost in oblika cvetnih lističev, vonj, čas cvetenja in skoraj neomejen izbor barvnih odtenkov.

Širok barvni spekter cvetov vrtnic je pogojen s prisotnostjo antocianinov, kvercetinov in karotenoidov v celicah (Eugster in Markifisher, 1991), ki skupaj s kopigmenti sodelujejo pri pigmentaciji (Davies in Mazza, 1993). V cvetovih rdeče in rožnato obarvanih sort vrtnic so najpogostejši antocianini derivati pelargonidina, cianidina in peonidina (Biolley in sod., 1994; Kumar in sod., 2008), med kvercetini prevladuje kvercetin-3-ramnozid (Cai in sod., 2005), med drugimi fenolnimi spojinami katehin, klorogenska kislina, galna kislina in protokatehulna kislina (Kumar in sod., 2008). Pelargonidin-3,5-diglukozid in cianidin-3,5-diglukozid sta prevladujoča antocianina pri rdečih sortah, potrjena je bila prisotnost številnih drugih antocianinov, predvsem pelargonidin-3-glukozida, cianidin-3-glukozida in

peonidin-3(5)-(di)-glukozida (Biolley in sod., 1994; Mikanagi in sod., 1995). V sodobnih požlahtnjenih sortah vrtnic prevladujejo antocianini z dvema sladkornima enotama (Mikanagi in sod., 1995)

Barva cvetov vrtnic je poleg pigmentov v vakuolah močno odvisna tudi od starosti venčnih listov. V procesu staranja zasledimo številne biokemijske in fiziološke spremembe, ki so značilne za posamezne faze cvetnega razvoja (Sood in sod., 2006). V točno določenem zaporedju se izvršijo procesi celične delitve, diferenciacije, sprememb v prepustnosti membrane, povečevanje celic in sinteza hormonov ter sekundarnih metabolitov, med njimi tudi pigmentov (Kumar in sod., 2008). Sood in Nagar (2003) sta izmerila povečanje vsebnosti fenolov od faze popka do polno odprtega cveta vrtnic *Rosa bourboniana* Desport in *Rosa damascena* Mill. Malo pa je znanega o spremembah pigmentov v fazah starajočega cveta. S staranjem cveta (senescenco) se namreč močno spremeni vsebnost skupnih fenolov v cvetovih vrtnic (Dela in sod., 2003), spremembe vsebnosti posameznih fenolov pa so bile do zdaj povsem neraziskane. Za vizualno spremembo in bledenje barve starajočih cvetov je predvidoma odgovorno zmanjšanje vsebnosti antocianinov v vakuolah. Poleg učinka razredčitve zaradi povečanja celic in celičnih organel, ki gotovo vpliva na dojemanje barve, je bil do sedaj opisan razpad antocianskih pigmentov pri tropski rastlini *Brunfelsia calycina* Benth. (Vaknin in sod., 2005) in v *Petunia* x *hybrida* cvetovih (Ferrante in sod., 2006), verjetno pa so podobni procesi prisotni tudi pri vrtnicah. Biosinteza fenolov, med njimi tudi antocianinov, je fazno pogojena, kot poročajo Noda in sod. (2004) pri *Eustoma grandiflora* (Raf.) Shinn. cvetovih in Sood in Nagar (2003) pri dveh vrstah vrtnic.

S staranjem cveta vrtnic (predvsem v zadnjih fazah razvoja cveta) je neposredno povezana sprememba pH vrednosti vakuolnega soka, ki se s senescenco veča kot poročajo Barthe in Valliant (1993) in Oren-Shamir in sod. (2001). Antociani so v vodnem okolju prisotni v štirih oblikah: flavilijevem kationu, kinonski bazi, karbinol psevdobazi in kalkonski bazi. Njihov delež je odvisen od pH vrednosti; v kislem okolju prevladuje rdeč flavilijev kation, s povečanjem pH vrednosti poteče hidroksilacija in razbarvanje, v bazičnem okolju pa prevladata modrikasta kinonska baza ali rumenkasta halkonska baza (Ougham in sod. 2005). Barva venčnih listov pri vrtnicah je odvisna od pH vrednosti celičnega soka; v dosedanjih raziskavah so izmerili spremembo pH vrednosti celičnega soka pri rezanih cvetovih rdeče sorte vrtnice, vendar biokemična sestava ni bila določena (Barthe in Vaillant, 1993). S staranjem se pH vrednost celičnega soka bistveno poveča, posledično se spremeni intenzivnost barvnega odtenka venčnega lista in prehod v modrikasto barvo.

Tudi pH vrednost substrata močno vpliva na dostopnost hranil (Papafotiou in sod., 2007) in kopičenje pigmentov (predvsem antocianov). Koncentracija karotenoidov v vakuolah venčnih listov pri *Impatiens* in *Petunia* hibridih je po raziskavi, ki so jo opravili Smith in sod. (2004), prav tako odvisna od pH vrednosti substrata. Miniaturne vrtnice so običajno sajene v rahlo kisle substratne mešanice (pH vrednost okoli 6), vendar pa ni podatkov o tvorbi antocianinov in barvi cvetov v različnih kislostih substrata.

Poskuse z miniaturnimi vrtnicami sorte 'KORcrisett' smo izvajali v rastlinjaku Biotehniške fakultete avgusta 2008. Sorta 'KORcrisett' je bila izbrana zaradi intenzivne svetlo rdeče barve in hitre tvorbe novih cvetov. V steklenjaku so bile dnevno nočne temperature nadzorovane s sistemom za hlajenje in se gibale med 27 °C podnevi in 22 °C ponoči. Relativna zračna vlažnost v steklenjaku je bila med 75 in 85 %, dodatna osvetlitev ni bila dovajana. Rastline so bile razporejene na poplavnih mizah po naključnem bločnem sistemu. Dnevno poplavljanje je bilo izvršeno ob isti uri v 4 minutnem poplavnem intervalu, temperatura vode je bila 18 °C. Kolorimetrične analize v poskusih in vzorčenjih v Arboretumu Volčji Potok so bile opravljene na sredini venčnega lista v treh ponovitvah na posameznem cvetu. Za kemične analize so bili vzorčeni le venčni listi (cvetovi brez čašnih listov, prašnikov in pestiča) v izbrani fazi razvoja.

V prvem poskusu smo merili spremembo barve in vsebnosti izbranih sekundarnih metabolitov v različno starih cvetovih vrtnic. Vzorčili smo cvetove v štirih fazah razvoja:

(1) Popek (cvetovi zaprti, pigmentacija popolna, čašni listi delno razprti);
(2) Delno odprt cvet (dva do tri zunanji venčni listi razprti, notranji del cvetne čaše zaprt);
(3) Popolnoma odprt cvet (venčni listi značilno razprti);
(4) Senescenten cvet (venčni listi razviti navzven, opazno razbarvanje, celoten cvet ob dotiku odpade).

V poskus je bilo vključenih 5 rastlin, na vsaki rastlini so bili vzorčene štiri faze cvetov v treh ponovitvah.

V drugem poskusu smo spremljali vpliv kislosti substrata na tvorbo novih cvetov in vsebnost izbranih sekundarnih metabolitov v venčnih listih. Vrtnice so bile sajene v 1,3 l lončke in tri substratne mešanice črne šote (80 v/v %) in kremenčevega peska (20 v/v %), ki smo jim z dodajanjem kalcijevega karbonata spremenili pH vrednost in določili tri obravnavanja:

(1) pH 3,3;
(2) pH 4,7;
(3) pH 7,3.

V drugem poskusu je bilo v vsako obravnavanje vključenih 5 rastlin, na posamezni rastlini smo vzorčili tri cvetove v fazi popolnoma odprtega cveta. Število vseh cvetov (od faze popka do senescentnega cveta) smo prešteli na začetku in ob koncu poskusa, v dveh terminih so bile prav tako opravljene tudi kemične analize vsebnosti sekundarnih metabolitov.

V avgustu 2009 smo v rožnem vrtu Arboretuma Volčji Potok vzorčili osem sort pokrovnih vrtnic; tri bele sorte ('NOAschnee', 'KOReb', 'Sea Foam'), tri rdeče sorte ('POUleas', 'MORedfar', 'Gärtnerfreude') in dve rožnati sorti ('KORverlandus', 'The Fairy'). Merili smo spremembe barve in vsebnosti izbranih fenolnih snovi v štirih fazah razvoja cveta, kot v

prvem poskusu na miniaturni vrtnici sorte 'KORcrisett'. V vsaki razvojni fazi je bilo vzorčenih pet cvetov na posamezni sorti.

Postavili smo naslednje raziskovalne hipoteze:

- Vsebnost specifičnih fenolnih snovi (antocianov, kvercetinov, fenolnih kislin) se spreminja s staranjem cvetov vrtnic;
- Intenzivnost barve venčnih listov s starostjo cveta upada;
- Izmerjeni barvni parametri so v tesni korelaciji z vsebnostjo antocianov v venčnih listih vrtnic;
- Vsebnost specifičnih fenolnih snovi je odvisna od pH substratne mešanice.

2 ZNANSTVENI ČLANKI

2.1 VPLIV TOPOFIZE NA SINTEZO ANTOCIANINOV PRI SORTAH PAHLJAČASTEGA JAVORJA (*Acer palmatum* THUNB.)

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Phase change modifies anthocyanin synthesis in *Acer palmatum* Thunb. (Japanese maple) cultivars.
Acta Physiologiae Plantarum, 2009, 31: 415-418.
Prejeto: 1. 9. 2008, sprejeto: 20. 10. 2008

Sposobnost koreninjenja lesnatih rastlin upada s starostjo matične rastline (ciklofiza), ob neugodnih okoljskih razmerah (perifiza) in glede na položaj veje/potaknjenca na drevnini (topofiza). Veje, ki izraščajo neposredno iz podlage ali nižje na provodniku kažejo juvenilne lastnosti, višje ležeče veje pa so fiziološko starejše. Le juvenilni deli rastline imajo dobro sposobnost razvoja nadomestnih (adventivnih) korenin, kar je zlasti opazno pri rastlinah, ki jih težje vegetativno razmnožujemo. Različne fiziološke, morfološke in histološke kazalce (markerje) juvenilnosti lahko dopolnimo tudi z biokemijskimi spremembami v posameznih delih lesnatih rastlin.

Preučevali smo vpliv položaja veje/lista na drevesu na količino antocianov. V jesenskem času smo pri sedmih sortah pahljačastih javorjev določili tri obravnavanja: (1) veja v vrhu krošnje, (2) srednje ležeča veja in (3) veja na spodnjem delu provodnika ter spremljali vsebnost rdečih pigmentov v listih. Vsebnosti posameznih antocianinov so bile določene s pomočjo tekočinske kromatografije visoke ločljivosti v kombinaciji z masno spektrometrijo (HPLC-MS). Pri vseh sortah pahljačastih javorjev smo potrdili prisotnost dveh prevladujočih antocianinov, cianidin-3-glukozida in cianidin-3-rutinozida, ter drugih derivatov cianidina in delfinidina v manjših količinah. Vsebnosti glavnih antocianinov so se močno razlikovale med sortami, razlike pa so se pokazale tudi ob primerjavi listov iz treh obravnavanj. Vsebnost cianidin-3-glukozida je bila vedno najvišja v listih, vzorčenih na vejah z vrha krošnje in najnižja v listih, ki so izraščali iz spodnjih vej. Tudi pri sortah, ki imajo rdeče obarvane liste, je bil potrjena enaka tendenca, zato je ta antocianin predstavljal kvantitativni biokemijski kazalec pozicijskega učinka pri pahljačastih javorjih. Vsebnost cianidin-3-rutinozida je bila pri večini sort največja v listih iz krošnje, vendar razmerje ni bilo tako očitno kot pri vsebnosti cianidin-3-glukozida.

Acta Physiol Plant (2009) 31:415–418
DOI 10.1007/s11738-008-0237-4

SHORT COMMUNICATION

Phase change modifies anthocyanin synthesis in *Acer palmatum* Thunb. (Japanese maple) cultivars

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Received: 1 September 2008 / Revised: 1 October 2008 / Accepted: 20 October 2008 / Published online: 21 November 2008

Abstract The potential markers of juvenility (cyanidin 3-glucoside and cyanidin 3-rutinoside) in autumn leaves of seven *Acer palmatum* Thunb. cultivars were investigated. Three shoot positions were marked on each cultivar—crown shoot, middle shoot, and basal shoot—and the anthocyanins were analyzed using HPLC-MS. The results showed great differences in cyanidin 3-glucoside and cyanidin 3-rutinoside concentrations among seven cultivars; moreover, significant differences in cyanidin 3-glucoside content levels were also observed among three shoot positions regardless of the cultivar analyzed. The concentration decreased basipetally and reached levels up to 52 times higher in leaves obtained from crown shoots in comparison to basal shoot leaves. Therefore, the content level of cyanidin 3-glucoside can be defined as a quantitative marker of positional effect in all the *Acer palmatum* Thunb. cultivars analyzed. The content level of cyanidin 3-rutinoside did not express the same positional dependence.

Keywords *Acer palmatum* Thunb. · Cultivars · Cyanidin 3-glucoside · Cyanidin 3-rutinoside · Positional effect

Introduction

Plant maturation is an extremely important horticultural characteristic, mostly because of its applicative influence on the production process. Specifically, local juvenility differences have significant consequences for the selection of plant parts for vegetative propagation and this is particularly critical in difficult-to-root plant species. It is widely known that the rooting ability in the majority of woody plants decreases with the chronological age of the stock plant (cyclophysis), when subjected to environmental stress (periphysis) and also acropetally—according to the position of the shoot in relation to the rest of the crown (topophysis). Only juvenile material is prone to developing strong and ample roots and/or axillary bud growth, which has recently been described in *Backhousia citriodora* F. Muell (Kibbler et al. 2003), *Dalbergia sissoo* Roxb. (Husen 2004), *Schlumbergera* 'Russian Dancer' (Kristiansen et al. 2005) and *Abies fraseri* (Pursh) Poir. (Rosier et al. 2005). Peer and Greenwood (2001) stressed the importance of the topophysis effect in *Larix* with a reference to the rooting percentage and root system quality. But are there any other markers beside the rooting ability that can efficiently describe the maturation stage of the plant material?

Many morphological, physiological, and histological parameters have previously been used to describe phase changes and topophysis in woody species, in order to assess the juvenility of plant material (Borchert 1976; Haffner et al. 1991). McGowran et al. (1998) evaluated morphological descriptors of plant juvenility (angle of shoots to the horizontal, shoot length, stem length, leaf area) in *Quercus robur* L. and *Quercus petraea* (Mattuschka) Liebl., Nicolini and Chanson (1999) described the differences in tree architecture in juvenile and mature stages of *Fagus sylvatica* L. trees, and leaf morphology was a decisive factor in research on genomic DNA methylation in *Acacia mangium* (Baurens et al. 2004). Biochemical parameters, such as variation in the carbohydrate content level, hormonal and polyphenol

Communicated by J. Zwiazek.

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synthesis, and enzymes have also been used to describe the change from juvenile to mature phase in hazelnut (Rey et al. 1994). However, very little is known about the hormonal regulation of the phase change in plants. Valdes et al. (2003, 2004) described the changes in hormonal status during maturation in *Pinus radiata* and *Pinus pinea*, where the mature phase appears to be characterized by inactivation of one or more enzymes involved in the biosynthesis of active polyphenols and flavonoids. In mature leaves of *Hedera helix* L. the inactivation of dehydroquercetin reductase (DQR) means that they do not accumulate anthocyanins (Haffner et al. 1991; Murray et al. 1994). On the other hand, anthocyanin accumulation in woody deciduous plants tends to be more advanced in mature leaves, causing a gradual autumn color from the crown to the inner parts of the plant. Is there a direct link between the stage of plant maturation and the level of anthocyanins in the leaves of *Acer palmatum* Thunb. cultivars? Positional effect may play a role in the anthocyanins content level and could bring a new quantitative approach to the study of the aging phenomenon in woody plants.

Materials and methods

Leaf samples of seven *Acer palmatum* Thunb. cultivars (*Acer palmatum* Thunb., 'Bloodgood', *A. p.* var. *palmatum* Thunb., 'Semi K2', 'Dissectum rubrifolium', 'Coreanum', and 'Dissectum nigrum') were obtained in the middle of October 2007 at three locations in the central region of Slovenia. All plants were growing in semishaded conditions, were mature and representative, not affected by pathogens, and with visible autumn color of the senescing leaves. Three positions on each cultivar were defined by vertical positioning according to the physiological maturity of woody plants (1. crown shoot, 2. middle shoot, and 3. base shoot) and five leaves were collected from each level, frozen in liquid nitrogen and stored at −18°C.

Single leaf samples were ground to a fine powder with liquid nitrogen and 0.2 g was extracted with 1 mL methanol containing 3% (v/v) HCOOH and 1% (w/v) 2,6-di-*tert*-butyl-4-methylphenol (BHT) in an ultrasonic bath for 1 h. After extraction, the treated samples were centrifuged for 7 min at 10,000 rpm. The supernatant was filtered through Chromafil AO-45/25 polyamide filter produced by Macherey-Nagel (Düren, Germany) and transferred to a vial prior to injection into the high-performance liquid chromatography (HPLC) system. The samples were analyzed using a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, USA) with a diode array detector at 530 nm. A Phenomenex HPLC column C18 (150 mm × 4.6 mm, Gemini 3μ) protected with a Phenomenex Security guard column, operated at 25°C, was used. The injection volume was 20 µL and the flow rate was 1 mL min^{-1}. The elution solvents were aqueous 1% formic acid (A) and Acetonitrile (B). The samples were eluted according to the linear gradient described by Marks et al. (2007). The concentrations of the cyanidin 3-glucoside and cyanidin 3-rutinoside were assessed from peak areas, quantified by the use of calibration curve of authentic standards and expressed as mg per kg fresh weight (FW). They were further identified using a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with an electroscopy interface (ESI) operating in positive ion mode using MS^2 scanning mode from *m/z* 115 to 800.

The results were statistically analyzed with the Statgraphics Plus program for Windows 4.0, using the one-way analysis of variance (ANOVA). The differences in the content levels of anthocyanins according to the position were estimated with Duncan's test. *P* values of less than 0.05 were considered statistically significant.

Results and discussion

Generally, the same anthocyanins were present in the leaves of all analyzed *Acer* cultivars, but there were significant differences in concentration according to the position of the shoots. The HPLC chromatograms obtained at 530 nm revealed two major peaks, which corresponded to the cyanidin 3-glucoside and the cyanidin 3-rutinoside and a number of minor peaks corresponding to cyanidin- and delphinidin-based anthocyanins. Several unidentified anthocyanins previously reported by Ji et al. (1991, 1992) and Fossen and Andersen (1999) were also observed. The major anthocyanin in the leaves of all *Acer* cultivars was cyanidin 3-glucoside, which is in accordance with the results of Harborne (1976) and Ishikura (1976); the highest level of this pigment was measured in the crown shoot leaves (419 mg 100 g^{-1}) and the lowest amount in leaves obtained from shoots growing at the base (8 mg 100 g^{-1}). In general, the concentration of cyanidin 3-glucoside was always lowest in leaves collected from the base shoots, slightly higher in the middle position, and highest in the leaves collected from the terminal shoots (Table 1). Cyanidin 3-rutinoside was continually present in small concentrations; the content level of this anthocyanin pigment was more or less stable, statistically significant differences in the concentrations according to shoot position were detected in *Acer palmatum* Thunb., *Acer palmatum* Thunb. *var. palmatum*, and 'Semi K2' (Table 2). Interestingly, the content level of cyanidin 3-rutinoside even increased from terminal to base position in 'Semi K2', which is the reverse trend compared to the case of cyanidin 3-glucoside in the *Acer* cultivars analyzed. The base/crown ratio in cyanidin 3-rutinoside concentration was therefore considerably lower—from 1:0.25 in 'Semi K2' to 1:2 in *Acer palmatum* Thunb.

Table 1 Cyanidin 3-glucoside content level (mg 100 g^{-1}) in the leaves of seven cultivars of *Acer palmatum* Thunb. from three positions

Acer palmatum Thunb. cultivar	Crown shoots	Middle shoots	Base shoots	Mean	Cyanidin 3-glucoside base/crown ratio
1	398 ± 20a	82 ± 9b	132 ± 81b	204 ± 45	1:3
2	419 ± 64a	121 ± 15b	8 ± 3b	183 ± 51	1:52
3	187 ± 10a	72 ± 9b	65 ± 11b	108 ± 16	1:3
4	178 ± 25a	56 ± 15b	11 ± 2b	82 ± 21	1:16
5	213 ± 12a	268 ± 34a	105 ± 19b	195 ± 22	1:2
6	167 ± 16a	83 ± 18b	47 ± 11b	99 ± 16	1:4
7	341 ± 47a	149 ± 13b	96 ± 11b	195 ± 32	1:4

Average values ± standard error are presented. Different letters (a, b) in rows mean statistically significant differences between cyanidin 3-glucoside contents from three branch positions at $\alpha < 0.05$ of one cultivar. The base to crown shoot ratio in concentration of cyanidin 3-glucoside in 7 *Acer palmatum* Thunb. cultivars are presented

1, *Acer palmatum* Thunb.; 2, *A. p.* var. *palmatum*; 3, *A. p.* 'Semi K2'; 4, *A. p.* 'Dissectum rubrifolium'; 5, *A. p.* 'Coreanum'; 6, *A. p.* 'Dissectum nigrum'; 7, *A. p.* 'Bloodgood'

Table 2 Cyanidin 3-rutinoside content level (mg 100 g^{-1}) in the leaves of seven cultivars of *Acer palmatum* Thunb. from three positions

Acer palmatum Thunb. cultivar	Crown shoots	Middle shoots	Base shoots	Mean	Cyanidin 3-rutinoside base/crown ratio
1	0.92 ± 0.07a	0.36 ± 0.02b	0.46 ± 0.16b	0.58 ± 0.08	1:2
2	3.46 ± 0.43a	1.36 ± 0.13b	1.53 ± 0.17b	2.12 ± 0.31	1:2
3	1.41 ± 0.36b	0.96 ± 0.14b	5.53 ± 1.07a	2.42 ± 0.62	1:0.25
4	0.56 ± 0.04a	0.54 ± 0.08a	0.52 ± 0.09a	0.54 ± 0.04	1:1
5	1.92 ± 0.21a	2.14 ± 0.26a	2.06 ± 0.13a	2.04 ± 0.11	1:1
6	8.38 ± 0.87a	5.38 ± 0.89a	9.64 ± 2.8a	7.75 ± 1.13	1:1
7	12.61 ± 0.41a	17.02 ± 4.02a	16.86 ± 2.22a	15.49 ± 1.53	1:1

Average values ± standard error are presented. Different letters (a, b) in rows mean statistically significant differences between cyanidin 3-rutinoside contents from three branch positions at $\alpha < 0.05$ of one cultivar. The base to crown shoot ratio in concentrations of cyanidin 3-rutinoside in seven *Acer palmatum* Thunb. cultivars are presented

1, *Acer palmatum* Thunb.; 2, *A. p.* var. *palmatum*; 3, *A. p.* 'Semi K2'; 4, *A. p.* 'Dissectum rubrifolium'; 5, *A. p.* 'Coreanum'; 6, *A. p.* 'Dissectum nigrum'; 7, *A. p.* 'Bloodgood'

Haffner et al. (1991) proposed that biochemical and molecular markers may complement the use of morphological markers in assessing the juvenility of plant material. Moreover, Fernandez-Lorenzo et al. (1999) analyzed 24 polyphenols as potential markers for differentiating juvenile and mature plant material in chestnut cultures, and a juvenile-phase-dependant accumulation of anthocyanin was observed in a number of woody perennials, including species of *Betula* (Brand and Lineberger 1992), and *Malus* (Noiton et al. 1992). In our study the cyanidin 3-glucoside concentration in maple leaves was significantly connected with the shoot position, since leaves from the top of the crown contained up to 52 times higher values compared with leaves from the tree base. The basipetally decreasing trend in cyanidin 3-glucoside concentration in our study was evident in all the *Acer palmatum* cultivars, which makes our results stronger. Moreover, even the cultivars that are red all year round had the same gradation tendency in cyanidin 3-glucoside concentrations; the leaves from crown shoots of 'Bloodgood' contained four times higher amounts of this pigment compared to leaves collected from base shoots.

The position on the plant apparently plays a role in the accumulation of the major anthocyanin in senescing leaves of *Acer palmatum* Thunb. cultivars analyzed. The data obtained in this study indicate that the level of cyanidin 3-glucoside in senescing leaves can be used as a quantitative marker of the maturation process. Because significant differences in cyanidin 3-glucoside concentration exist among three shoot positions on the mature plant, it will be of further interest to investigate the art of cyanidin 3-glucoside accumulation in the rejuvenated plants (cuttings, micropropagated plants). Such experiments can help us further understand the specific metabolic pathways of anthocyanins and phase-specific anthocyanin accumulation, significant for the understanding of the positional effect in woody plants.

Acknowledgments This work is part of Horticulture Program No. P4-0013-0481, granted by the Slovenian Ministry for Higher Education, Science and Technology.

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2.2 POVEZAVA MED BARVNIMI PARAMETRI IN PREVLADUJOČIMI ANTOCIANINI PRI SEDMIH SORTAH *Acer palmatum* THUNB.

SCHMITZER Valentina, OSTERC Gregor, VEBERIČ Robert in ŠTAMPAR Franci
Correlation between chromaticity values and major anthocyanins in seven *Acer palmatum* Thunb. cultivars.
Scientia Horticulturae, 2009, 119: 442-446.
Prejeto: 7. 4. 2008, sprejeto: 2. 9. 2008

Pahljačasti javorji so priljubljeni v javnih in zasebnih nasadih, predvsem zaradi intenzivne obarvanosti listja v jesenskem času. V raziskavi smo povezali barvne parametre, izmerjene s prenosnim kolorimetrom, z vsebnostjo antocianinov v listih sedmih sort pahljačastih javorjev, *Acer palmatum* Thunb., *A. p.* var *palmatum*, *A. p.* 'Semi K2', *A. p.* 'Dissectum rubrifolium', *A. p.* 'Dissectum nigrum', *A. p.* 'Coreanum' in *A. p.* 'Bloodgood'. Določili smo tri obravnavanja glede na položaj veje na drevesu: (1) veja z vrha krošnje, (2) srednje ležeča veja in (3) veja na spodnjem delu krošnje. Parameter *a** (-60 do 60, od zelene do rdeče) je bil pri vseh sortah največji v (1) obravnavanju in najmanjši v (3) obravnavanju, močno pa se je razlikoval tudi med sortami. S pomočjo tekočinske kromatografije visoke ločljivosti smo v listih pahljačastih javorjev ugotovili prisotnost dveh antocianinov: prevladujočega cianidin-3-glukozida in manj zastopanega cianidin-3-rutinozida. Največje vrednosti cianidin-3-glukozida so bile izmerjene v listih pahljačastih javorjev v (1) obravnavanju, ugotovljene so bile tudi razlike med sortami. Pri vseh sortah so bile potrjene tesne korelacije med parametrom *a** in vsebnostjo cianidin-3-glukozida, prav tako so bile izmerjene močne povezave med vsebnostjo prevladujočega antocianina in kolorimetričnimi parametri *b** (-60 do 60, od modre do rumene), *h*° (kot med 0 in 360°, ki določa barvo) in *L** (0 do 100, od svetle do temne). Korelacije med manj zastopanim cianidin-3-rutinozidom in barvnimi parametri niso bile tesne pri vseh sortah. Izražanje rdeče barve pri pahljačastih javorjih je tako močno povezano z vsebnostjo prevladujočega antocianina v listih, manj pa z vsebnostjo drugih izmerjenih rdečih pigmentov.

Scientia Horticulturae 119 (2009) 442–446

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Correlation between chromaticity values and major anthocyanins in seven *Acer palmatum* Thunb. cultivars

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ARTICLE INFO

Article history:
Received 7 April 2008
Received in revised form 28 August 2008
Accepted 2 September 2008

Keywords:
Acer palmatum Thunb. cultivars
Autumn color
Anthocyanins
Correlation

ABSTRACT

The correlation between the anthocyanin concentrations of seven *Acer palmatum* Thunb. cultivars and the chromaticity values a^*, b^*, a^*/b^* ratio, h° and L^* was investigated. According to the position of leaves on the branch terminal, middle and base sample leaves were analyzed and the positional effect on the level of the major anthocyanin was estimated. Leaf color was measured with a portable colorimeter and individual anthocyanins were detected with the use of HPLC–MS. Cyanidin-3-glucoside was present in all senescing leaves of *Acer* cultivars and its content decreased from terminal to base position. Cyanidin-3-rutinoside was identified in lower concentrations in the leaves of all cultivars. Multiple variable analysis was calculated for each of the tristimulus values and two major anthocyanins (cyanidin-3-glucoside, cyanidin-3-rutinoside) in senescing leaves of ornamental *Acer* cultivars. Correlations were detected between the major anthocyanin (cyanidin-3-glucoside) in analyzed *Acer* cultivars and all chromaticity parameters. The highest correlation coefficient (0.94) was observed between cyanidin-3-glucoside and a^*/b^* ratio in cultivar 'Bloodgood' and lowest (0.54) between cyanidin-3-glucoside and a^* in *Acer palmatum* Thunb. The correlation between cyanidin-3-rutinoside and chromaticity parameters was not detected in all *Acer* cultivars, additionally correlation coefficients and statistical significance were much lower. The expression of red color in both senescing leaves and in all-year-red cultivars can be tightly linked with the content level of cyanidin-3-glucoside; the second major anthocyanin does not contribute much to the red leaf color.

1. Introduction

The genus *Acer* (*Aceraceae*) contains approx. 200 species, widely distributed in the northern hemisphere and planted as ornamentals all over the World (Ji et al., 1992). *Acer palmatum* Thunb. (Japanese maple) is a popular decorative plant with many cultivars praised for the all year reddish color of its leaves and/or spectacular autumn colors, which makes it a widely used plant in parks, urban spaces and gardens. The visual attributes of the genus *Acer* are most evident in autumn, when the color display ranges from bright orange to dark purple.

The expression of red color in the leaves of *Acer rubrum* Thunb., *Cercis canadensis* L. and *Liquidambar styraciflua* L. strongly correlates with the presence of anthocyanins in the vacuoles of epidermal and/or mesophyll cells (Hughes et al., 2007). The level of anthocyanin content in leaves varies with genotype, cultural practices and environmental factors (Jakopic et al., 2007). Often as a response to stress such as cold, high light levels, deficiency of nutrients and pathogen attack (Davies, 2004). Pigment composition in leaves, particularly the loss of chlorophyll and accumulation of anthocyanins can also differ with physiological maturity of woody plants. This can be observed in the gradual coloration of woody plants in autumn, according to the position of leaves on shoots.

Anthocyanin leaf analysis of deciduous plants is commonly carried out using high-performance liquid chromatography (HPLC). Fossen and Andersen (1999) extracted various anthocyanin pigments from the leaves of *Acer platanoides* 'Crimson King'. Similarly, Olszevska (2007) extracted flavonoids from *Prunus serotina* leaves. The main anthocyanins in senescing *Acer* sp. leaves are cyanidin-3-glucoside and cyanidin-3-rutinoside, other cyanidin and delphinidin based anthocyanins are present only in small amounts in some cultivars (Ji et al., 1991; Ji et al., 1992; Fossen and Andersen, 1999). Plant tissue color is easily obtained with a portable colorimeter; moreover, the parameters represent an objective measure of the specific color. Several studies show that there is a correlation between the predominant type of anthocyanin and one or more chromatic parameters in *Myricia Jaboticaba*

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doi:10.1016/j.scienta.2008.09.003

Berg. fruit pulp (Montes et al., 2005), petals of the flowering lotus (Katori et al., 2002) and the skin of 'Delicious' apple (Singha et al., 1991). A linear relationship between anthocyanin concentration and hue value h° was also obtained in results of Lancaster et al. (1997), who studied the influence of pigment composition on skin color in a wide range of fruit and vegetables.

The aim of this study was to determine the relationship between any of the tristimulus values and the major anthocyanin in *Acer palmatum* Thunb. cultivars. Additionally, we studied the differences in concentration of the major anthocyanin as affected by leaf position on the branches (terminal, middle and base position). Is the role of physiological maturation on pigment composition evident in all studied *Acer palmatum* Thunb. cultivars? Do all cultivars tend to have the same patterns in the autumn coloring of leaves according to the position of the leaves on branches? Although some authors have determined single anthocyanins in autumn leaves of various maple species, differences in concentration of specific anthocyanins in ornamental cultivars of *Acer palmatum* Thunb. have never previously been assessed. The visual evaluation of the popular cultivars can be upgraded with a colorimetric analysis and furthermore linked to the major anthocyanins in the senescing leaves of ornamental plants. This can be used to predict the concentration of the major anthocyanin in leaves of ornamental *Acer* cultivars and consequently select the potential breeding material for new cultivars with intense color of the leaves.

2. Materials and methods

2.1. Plant material

Seven cultivars of *Acer palmatum* Thunb. species (*Acer palmatum* Thunb., *A. p.* var *palmatum*, *A .p.* 'Semi K2', *A. p.* 'Dissectum rubrifolium', *A. p.* 'Coreanum', *A. p.* 'Dissectum nigrum' and *A. p.* 'Bloodgood') were collected in the middle of October 2007 from three locations in the central region of Slovenia. *Acer palmatum* Thunb. sample leaves were obtained from the Ljubljana Botanical garden, *A. p.* 'Bloodgood' from the private nursery (Ziher Spur) and the remaining cultivars from the Volcji potok arboretum. All plants were mature and representative of the cultivar, growing in similar, semishaded conditions. Three branch positions were determined on every plant according to the physiological maturity of woody plants: (1) terminal, (2) middle and (3) base. From each plant, 15 sample leaves were collected, 5 for each position on the branch. The color parameters of each leaf were measured with a portable colorimeter, then frozen in liquid nitrogen and lyophilized. Samples were stored at −18 °C until further analysis.

2.2. Leaf color measurements

Leaf color was measured using the Minolta CR-10 Chroma portable colorimeter (Minolta Co., Osaka, Japan) with C illuminant. The colorimeter was calibrated with a white standard calibration plate before use. In this system of color representation, the L^* value corresponds to a dark–bright scale and represents the relative lightness of colors with a range from 0 to 100 (0 = black, 100 = white). The a^* and b^* values extend from −60 to 60; a^* negative is for green and a^* positive is for red and b^*negative is for blue and positive for yellow. The hue angle (h°) is expressed in degrees from 0 to 360, where 0° = red, 90° = yellow, 180° = green and 360° = blue. Color was measured in the middle of each leaf sample to ensure equal measurement conditions. Fifteen samples were measured for each cultivar. Dissectum type leaves (*A. p.* 'Dissectum rubrifolium' and *A. p.* 'Dissectum nigrum') were excluded from this part of analysis because acquiring comparable results was not possible, owing to the small size of leaves and extensive nervature.

2.3. Extraction method and HPLC analysis of anthocyanins

For the analysis of single anthocyanins, frozen leaf samples were ground to a fine powder with liquid nitrogen. A sample of 0.2 g from each leaf was extracted with 1 mL methanol containing 3% (v/v) HCOOH and 1% (w/v) 2,6-di-*tert*-butyl-4-methylphenol (BHT) in an ultrasonic bath for 1 h. After extraction, the treated samples were centrifuged for 7 min at 10,000 rpm. The supernatant was filtered through Chromafil AO-45/25 polyamide filter (Macherey-Nagel Düren, Germany) and transferred to a vial prior to injection into the high-performance liquid chromatography (HPLC) system.

The samples were analyzed using a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, USA) with a diode array detector at 530 nm. A Phenomenex HPLC column C18 (150 mm × 4.6 mm, Gemini 3μ) protected with a Phenomenex Security guard column operated at 25 °C was used. The injection volume was 20 μL and the flow rate was 1 mL min^{-1}. The elution solvents were aqueous 1% formic acid (A) and acetonitrile (B). The samples were eluted according to the linear gradient described by Marks et al. (2007).

The anthocyanins were identified using a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with an electroscopy interface (ESI) operating in positive ion mode. Analysis of anthocyanins was carried out using MS^2 scanning mode from m/z 115 to 800.

2.4. Chemicals

The standards used to determine the anthocyanins in samples were kuromanin chloride (cyanidin-3-*O*-glucoside chloride) and keracyanin chloride (cyanidin-3-*O*-rutinoside chloride) from Sigma–Aldrich Chemie GmbH (Steinheim, Germany).

The chemicals for sample preparation and mobile phases were methanol, 2,6-di-*tert*-butyl-4-methylphenol (BHT) and acetonitrile from Sigma–Aldrich Chemie GmbH (Steinheim, Germany) and formic acid from Fluka Chemie GmbH (Buchs, Switzerland). The water used in solutions was bidistilled and purified with a Milli-Q water purification system by Millipore (Bedford, MA).

2.5. Statistical analysis

The results were statistically analyzed with the Statgraphics Plus program for Windows 4.0, using the one-way analysis of variance (ANOVA). Differences in content levels of both the anthocyanins and color variables were estimated with Duncan's test. *P*-values of less than 0.05 were considered statistically significant. Regression analysis was calculated for cyanidin-3-glucoside, cyanidin-3-rutinoside and each of the color variables estimated with a portable colorimeter.

3. Results and discussion

3.1. Measurements of leaf color

The results of the analysis of association between pigmentation parameters and the position of foliage on the branches is presented in Table 1. The results show a negative association between foliage position, specifically the shoots from terminal to base of the trunk, with each of the parameters, a^* and a^*/b^* ratio. A positive trend to the base of the trunk is apparent in the b^*, L^* and h° values. The a^* value ranged from 41.0 in the terminal position of *Acer palmatum*

Table 1
Leaf color measurements of five cultivars of *Acer palmatum* Thunb. on three branch positions

Acer palmatum Thunb. cultivar and position	a^*	b^*	a^*/b^* ratio	L^*	h°
1, t	41.0 ± 2.3 a	11.0 ± 0.3 a	3.7 ± 0.2 a	27.4 ± 0.4 a	15.2 ± 0.7 a
1, m	32.8 ± 1.6 b	37.2 ± 2.9 b	0.9 ± 0.1 b	46.5 ± 1.9 b	49.3 ± 3.4 b
1, b	22.7 ± 2.3 c	45.0 ± 3.9 b	0.4 ± 0.0 c	52.6 ± 2.7 c	62.6 ± 4.5 c
2, t	10.5 ± 1.6 a	3.3 ± 0.4 a	3.3 ± 0.4 a	19.5 ± 1.1 a	18.1 ± 2.1 a
2, m	1.7 ± 1.8 b	9.4 ± 1.3 b	0.1 ± 0.2 b	26.9 ± 1.3 b	77.2 ± 11.7 b
2, b	−10.6 ± 0.4 c	22.4 ± 0.5 c	−0.5 ± 0.0 b	36.0 ± 0.6 c	115.4 ± 0.7 c
3, t	24.5 ± 2.0 a	9.5 ± 2.0 a	2.7 ± 0.4 a	24.8 ± 0.5 a	24.9 ± 4.1 a
3, m	10.1 ± 3.9 b	23.3 ± 2.9 b	0.5 ± 0.2 b	34.3 ± 2.2 b	64.7 ± 9.8 b
3, b	11.0 ± 1.4 b	25.8 ± 2.0 b	0.5 ± 0.1 b	36.7 ± 0.7 b	66.2 ± 4.0 b
5, t	10.1 ± 0.6 a	5.2 ± 0.6 a	2.1 ± 0.3 a	23.2 ± 11.0 a	27.1 ± 3.0 a
5, m	10.4 ± 0.9 a	6.9 ± 0.5 a	1.8 ± 0.3 a	23.8 ± 0.9 a	30.6 ± 4.3 a
5, b	2.8 ± 1.0 b	16.0 ± 1.7 b	0.2 ± 0.1 b	30.4 ± 0.7 b	78.4 ± 4.5 b
7, t	15.7 ± 0.8 a	4.7 ± 0.5 a	3.5 ± 0.5 a	24.7 ± 0.5 a	17.1 ± 2.4 a
7, m	10.4 ± 0.7 b	7.1 ± 1.2 ab	1.8 ± 0.2 b	26.4 ± 1.0 ab	29.4 ± 2.5 b
7, b	12.1 ± 0.5 b	9.0 ± 0.8 b	1.3 ± 0.2 b	27.2 ± 0.8 b	38.7 ± 4.1 b

1, *Acer palmatum* Thunb.; 2, *A. p.* var. *palmatum*; 3, *A. p.* 'Semi K2'; 5, *A .p.* 'Coreanum'; 7, *A .p.* 'Bloodgood'.
t, terminal position of leaf on branch; m, middle position of leaf on branch; b, base position of leaf on branch. Average values ± standard error are presented. Different letters (a–c) in columns of each individual cultivar denote statistically significant differences between tristimulus color measurements and three leaf positions on the branch at $\alpha < 0.05$.

Thunb. to −10.6 for leaves on branch base of *A. p.* var. *palmatum*. There are significant differences in a^* value between leaves at branch base and those on terminal positions in all cultivars analyzed; leaves are redder on terminal positions of all cultivars analyzed. All b^* values were positive for all positions. The highest b^* value of 45.0 was observed in the leaves of *Acer palmatum* Thunb. at the base position, while the lowest value of 3.3 was observed at the terminal position of *A. p.* var. *palmatum*. Significant differences were observed in b^* value between leaves at branch base and those on terminal positions in all cultivars analyzed. The magnitude of lightness (L^*) and hue (h°), for most of the cultivars, decreases form branch base to the terminal position, as red leaves tend to be darker than the greener ones. However, this trend is less apparent in the all-year-red-leaf cultivars 'Coreanum' and 'Bloodgood'. Lightness ranged from 52.6 in *Acer palmatum* Thunb. to a darker 19.5 in *Acer palmatum* var. *palmatum*. According to the branch position (terminal/base), significant differences in L^* value were observed in all cultivars analyzed. With respect to hue (h°), the values ranged from 115.4 in *A. p.* var. *palmatum* to 15.2 in the leaves of *Acer palmatum* Thunb., with significant positional differences found in all cultivars. The a^*/b^* ratio values ranged from −0.5 in the base position of *A. p.* var. *palmatum* to 3.7 in terminal position of *Acer palmatum* Thunb. Significant differences in a^*/b^* ratio values between leaves at branch base and those at terminal positions were observed among all cultivars analyzed. Leaves of most *Acer palmatum* Thunb. cultivars tend to have more red (higher a^* values) and less yellow (lower b^* values) coloration at the terminal positions and become less vibrant and more yellow–green towards the trunk of the plant as indicated by lower a^* and higher b^* values, respectively. In cultivars 'Coreanum' and 'Bloodgood', whose leaves are red all-year-round a less apparent positional variation is detected. The magnitude of the difference in b^* values is relatively smaller in the senescing leaves of cultivars 'Coreanum' and 'Bloodgood' than that of other cultivars.

3.2. Cyanidin-3-glucoside content

The results of the analysis of the content of the major anthocyanin in the leaves of different *Acer* cultivars are presented in Table 2. The most abundant anthocyanin in autumn leaves of *Acer palmatum* Thunb. cultivars in our study was cyanidin-3-glucoside. This is in accordance with the results of Harborne (1976) and Ishikura (1976), who determined cyanidin-3-glucoside the most common anthocyanin in senescing leaves of angiosperms. The highest level (4.19 mg g^{-1}) of this pigment was observed in *Acer palmatum* var. *palmatum* at the terminal position, followed by *Acer palmatum* Thunb. leaves (3.90 mg g^{-1}) at the same position. The lowest amount (0.08 mg g^{-1}) of cyanidin-3-glucoside was found in *Acer palmatum* var. *palmatum* at the base position. Significant differences in the cyanidin-3-glucoside content were observed between leaves at the branch base and those at terminal positions among all cultivars analyzed. In general, the highest amount of the major anthocyanin was observed in senescing leaves at the terminal position, regardless of cultivar, and the lowest anthocyanin content was observed in leaves from the base branch position. Cyanidin-3-glucoside content level of leaves in the middle of branch position compared to that at the base position varied considerably; however, except in cultivar 'Coreanum'

Table 2
Cyanidin-3-glucoside content in leaves of 7 cultivars of *Acer palmatum* Thunb. on three branch positions

Acer palmatum Thunb. cultivar	Cyanidin-3-glucoside (mg g^{-1})			
	Terminal position	Middle position	Base position	Mean
1	3.98 ± 0.20 a	0.82 ± 0.09 b	1.32 ± 0.81 b	2.04 ± 0.45
2	4.19 ± 0.64 a	1.21 ± 0.15 b	0.08 ± 0.03 b	1.83 ± 0.51
3	1.87 ± 0.10 a	0.72 ± 0.09 b	0.65 ± 0.11 b	1.08 ± 0.16
4	1.78 ± 0.25 a	0.56 ± 0.15 b	0.11 ± 0.02 b	0.82 ± 0.21
5	2.13 ± 0.12 a	2.68 ± 0.34 a	1.05 ± 0.19 b	1.95 ± 0.22
6	1.67 ± 0.16 a	0.83 ± 0.18 b	0.47 ± 0.11 b	0.99 ± 0.16
7	3.41 ± 0.47 a	1.49 ± 0.13 b	0.96 ± 0.11 b	1.95 ± 0.32

1, *Acer palmatum* Thunb.; 2, *A. p.* var. *palmatum*; 3, *A. p.* 'Semi K2'; 4, *A .p.* 'Dissectum rubrifolium'; 5, *A .p.* 'Coreanum'; 6, *A. p.* 'Dissectum nigrum'; 7, *A .p.* 'Bloodgood'. Average values ± standard error are presented. Different letters (a, b) in rows mean statistically significant differences between cyanidin-3-glucoside contents on three branch positions at $\alpha < 0.05$.

Table 3
Correlation coefficients between chromaticity parameters a^*, b^*, a^*/b^* ratio, C^*, h° and L^* and cyanidin-3-glucoside in five cultivars of *Acer palmatum* Thunb.

Acer palmatum Thunb. cultivars	a^*	b^*	a^*/b^* ratio	h°	L^*
1	0.54 *	−0.68 **	0.78 ***	−0.68 **	−0.67 **
2	0.88 ***	−0.80 ***	0.87 ***	−0.88 ***	−0.83 ***
3	0.80 ***	−0.92 ***	0.93 ***	−0.92 ***	−0.90 ***
5	0.91 ***	−0.82 ***	0.82 ***	−0.86 ***	−0.83 ***
7	0.89 ***	−0.70 **	0.94 ***	−0.81 **	−0.67 *

1, *Acer palmatum* Thunb.; 2, *A. p.* var. *palmatum*; 3, *A. p.* 'Semi K2'; 5, *A .p.* 'Coreanum'; 7, *A .p.* 'Bloodgood'.
Correlation coefficients between each chromaticity value and cyanidin-3-glucoside with statistically significant differences of the estimated correlations presented as $^*\alpha < 0.05$, $^{**}\alpha < 0.01$ and $^{***}\alpha < 0.001$, NS not significant.

statistically significant difference from the base position was not detected. The high amount of anthocyanins in leaves at the terminal branch position could be explained by higher photosynthetic activity of the outer leaves relative to those in the shade (i.e. those in the middle and at the base branch positions). Consequently, leaves in terminal branch positions have higher nutrient concentrations, leading to elevated formation of secondary metabolites, including anthocyanins. It was previously suggested (Ishikura, 1976) that the accumulation of sugars in aged leaves facilitates a vigorous formation of anthocyanin during autumn senescence.

The second major anthocyanin in all cultivars was identified as cyanidin-3-rutinoside, the highest level (0.17 mg g^{-1}) being measured in the leaves of *Acer palmatum* 'Bloodgood' in the middle and base position and lowest (0.01 mg g^{-1}) in the *Acer palmatum* Thunb. leaves in middle position (data not shown). Results showed the presence of other cyanidin and delphinidin based anthocyanins in leaf samples of all cultivars analyzed, their concentrations were up to 1000 times lower than that of cyanidin-3-glucoside and no correlation with the tristimulus values was observed. Similar results on the anthocyanin content levels were reported in works of Ji et al. (1991, 1992) and Fossen and Andersen (1999).

3.3. Correlation between anthocyanin levels and tristimulus color measurements

Significant correlations between cyanidin-3-glucoside and all tristimulus values were observed in all *Acer palmatum* Thunb. cultivars analyzed (Table 3). A high correlation coefficient was observed between the major anthocyanin and a^*, with values ranging from 0.54 in *Acer palmatum* Thunb. to 0.91 in cultivar 'Coreanum'. Except for *Acer palmatum* Thunb. ($\alpha < 0.05$), a very high statistical significance of the estimated correlations ($\alpha < 0.001$) was observed in all cultivars. This demonstrates a tight connection between the amount of cyanidin-3-glucoside and the parameter a^*, suggesting this anthocyanin indeed being primarily responsible for the expression of red color in senescing leaves of all cultivars analyzed. Correlation coefficients between cyanidin-3-glucoside and b^* were all negative and ranged from −0.68 in *Acer palmatum* Thunb. to −0.92 in 'Semi K2'. Highly significant correlation was obtained also from a^*/b^* ratio and cyanidin-3-glucoside. It was most evident in cultivar 'Bloodgood' (0.94) and 'Semi K2' (0.93) and lowest in *Acer palmatum* Thunb. (0.78). Correlation coefficients between cyanidin-3-glucoside and the parameter h° were negative for all cultivars. High correlations were observed between h° and cyanidin-3-glucoside, particularly in cultivars 'Semi K2' (−0.92), *Acer palmatum* var. *palmatum* (−0.88) and 'Coreanum' (−0.86) with very high statistical significance ($\alpha < 0.01$ and $\alpha < 0.001$). Highly significant correlations were also observed between cyanidin-3-glucoside and the parameter L^*, the negative values ranging from −0.67 in *Acer palmatum* Thunb. and cultivar 'Bloodgood' to −0.90 in 'Semi K2'.

Additionally, the correlation coefficient between all tristimulus values and the second major anthocyanin, cyanidin-3-rutinoside, was calculated but could not be generalized to all cultivars (Table 4). However, correlations between cyanidin-3-rutinoside and a^* were observed in *Acer palmatum* var. *palmatum* (0.54) and 'Bloodgood' (0.59) but were much lower than those between cyanidin-3-glucoside and a^*. Significant correlations were also observed between cyanidin-3-glucoside and b^* (*Acer palmatum* Thunb., −0.62), a^*/b^* ratio (*Acer palmatum* Thunb., 0.72 and *Acer palmatum* var. *palmatum*, 0.66), h° (*Acer palmatum* Thunb., −0.62 and *Acer palmatum* var. *palmatum*, −0.62) and L^* (*Acer palmatum* Thunb., −0.61). Leaf samples of all *Acer* cultivars contained only small amounts of cyanidin-3-rutinoside; therefore, the results vary considerably, making statistical analysis less accurate. Consequently, the cyanidin-3-rutinoside content level in senescing leaves of *Acer palmatum* Thunb. cultivars does not correlate with tristimulus color measurements and cannot be directly linked to red autumn display in most of the *Acer* cultivars studied.

Table 4
Correlation coefficients between chromaticity parameters a^*, b^*, a^*/b^* ratio, C^*, h° and L^* and cyanidin-3-rutinoside in five cultivars of *Acer palmatum* Thunb.

Acer palmatum Thunb. cultivars	a^*	b^*	a^*/b^* ratio	h°	L^*
1	0.47 NS	−0.62 *	0.72 **	−0.62 *	−0.61 *
2	0.54 *	−0.40 NS	0.66 **	−0.620*	−0.51 NS
3	−0.13 NS	0.22 NS	−0.29 NS	0.21 NS	0.37 NS
5	0.11 NS	0.08 NS	−0.17 NS	0.07 NS	−0.02 NS
7	−0.59 *	0.09 NS	−0.42 NS	0.31 NS	−0.01 NS

1, *Acer palmatum* Thunb.; 2, *A. p.* var. *palmatum*; 3, *A. p.* 'Semi K2'; 5, *A .p.* 'Coreanum'; 7, *A .p.* 'Bloodgood'.
Correlation coefficients between each chromaticity value and cyanidin-3-rutinoside with statistically significant differences of the estimated correlations presented as $^*\alpha < 0.05$, $^{**}\alpha < 0.01$ and $^{***}\alpha < 0.001$, NS not significant.

4. Conclusions

The major anthocyanin in all seven ornamental *Acer* cultivars was cyanidin-3-glucoside; its level decreased from terminal to base positions of leaves on branches. The visual change, presented with the tristimulus values, from greener leaves in the base (lower a^* and a^*/b^* ratio, higher b^*, L^* and h°) to red leaves at the terminal positions (higher a^* and a^*/b^* ratio, lower b^*, L^* and h°) was directly linked to the amount of cyanidin-3-glucoside. Statistically significant correlations were detected between all tristimulus values and the major anthocyanin in the senescing leaves of seven *Acer* cultivars analyzed. Correlations were also observed between the second major anthocyanin cyanidin-3-rutinoside and tristimulus values but varied significantly among cultivars.

The data on major anthocyanins, responsible for the attractive color in selected ornamental cultivars, are important for the future breeding and further research on the influence of different environmental factors and genotype on the anthocyanin production in ornamentals. The positional effect on the anthocyanin synthesis in the connection to the adventitious root formation in ornamental *Acer* cultivars will be the field of research in future studies.

Acknowledgement

This work is part of the Horticulture Program P4-0013-0481 supported by the Slovenian Ministry of Higher Education, Science and Technology.

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2.3 SPREMEMBE VSEBNOSTI FENOLOV MED RAZVOJEM CVETA PRI VRTNICI 'KORcrisett'

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Changes in the phenolic concentration during flower development of rose 'KORcrisett'
Journal of the American Society for Horticultural Science, 2009, 134: 491-496.
Prejeto: 19. 6. 2009, sprejeto: 17. 7. 2009

Staranje cvetov pri miniaturnih vrtnicah je neposredno povezano z vizualno spremembo barve. Pri rdečih sortah vrtnic so na posamezni rastlini v primerjavi s starejšimi cvetovi popki obarvani intenzivneje. Spremembe do sedaj niso bile opisane s kolorimetričnimi meritvami ali fenolnim profilom venčnih listov.

S pomočjo tekočinske visokoločljivostne kromatografije smo preučevali spremembe vsebnosti antocianinov, kvercetinov, katehina in fenolnih kislin pri starajočih cvetovih vrtnice *Rosa* x *hybrida* 'KORcrisett'. Obenem smo izmerili kromatografske barvne parametre in izračunali korelacije med njimi in prevladujočimi ter skupnimi antocianini. Na posamezni rastlini smo določili štiri obravnavanja/razvojne faze cvetov: (1) popek, (2) delno odprt cvet, (3) popolnoma odprt cvet in (4) senescenten cvet. Z razvojem cveta smo opazili zmanjšanje vrednosti barvnega parametra *a**, ki opisuje količino rdeče barve v vzorcu, ter povečanje vrednosti parametra *L**, ki opredeli svetlost. Prav tako je bilo z razvojem cveta izmerjeno zmanjšanje vrednosti parametra *b**, kar predstavlja premik k modrikastim odtenkom venčnih listov pri senescentnih cvetovih. V venčnih listih vrtnice 'KORcrisett' je bila potrjena prisotnost dveh prevladujočih antocianinov, pelargonidin-3,5-di-glukozida in cianidin-3,5-di-glukozida, ter treh manj zastopanih antocianinov, pelargonidin-3-glukozida, cianidin-3-glukozida in peonidin-3-glukozida. Vsebnost vseh opazovanih antocianinov se je zmanjševala z razvojem cveta; koncentracija prevladujočih antocianinov je bila trikrat višja v popkih kot v senescentnih cvetovih. Prav tako je bil z razvojem cveta opazen upad vsebnosti kvercetinov (kvercetin-3-rutinozida, kvercetin-3-glikozida in kvercetin-3-ramnozida), katehina in fenolnih kislin (galne kisline, protokatehulne kisline, klorogenske kisline, kavine kisline, *p*-kumarne kisline) v venčnih listih vrnice 'KORcrisett'. Sprememba vsebnosti galne kisline je bila najbolj opazna, saj so popki vsebovali šestkrat več te fenolne kisline kot senescentni cvetovi. Barvni parametri *a**, *b**, *a**/*b**, *h*° in *L** so bili v tesni korelaciji z vsebnostjo prevladujočih in skupnih antocianinov v venčnih listih. Vizualne spremembe starajočih cvetov rdeče sorte vrtnice 'KORcrisett' so bile dopolnjene s spremembami fenolne sestave.

J. Amer. Soc. Hort. Sci. 134(5):491–496. 2009.

Changes in the Phenolic Concentration during Flower Development of Rose 'KORcrisett'

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Additional index words. *Rosa* ×*hybrida*, stages, color measurements, anthocyanins, phenolics, correlation

Abstract. **The concentration of major anthocyanins, quercetins, catechin, and phenolic acids during flower development of *Rosa* ×*hybrida* L. 'KORcrisett' was quantified using high-performance liquid chromatography/mass spectrometry. Additionally, the changes in petal color were monitored colorimetrically at four different stages of development (bud, partially open flowers, fully open flowers, senescent flowers) and correlation was calculated between the chromaticity parameters and major/total anthocyanins. Color parameters *a**, *b**, and *h*° decreased with the progression of flower development and *a**/*b** ratio and lightness (*L**) increased. In rose petals, a negative trend in the content of major (pelargonidin-3,5-di-O-glucoside, cyanidin-3,5-di-O-glucoside) and minor (pelargonidin-3-O-glucoside, cyanidin-3-O-glucoside, peonidin-3-O-glucoside) anthocyanins was observed during flower development. Buds contained almost threefold higher concentrations of pelargonidin-3,5-di-O-glucoside and fourfold higher concentrations of cyanidin-3,5-di-O-glucoside than senescent flowers. Buds also contained significantly more quercetins (quercetin-3-O-rutinoside, quercetin-3-O-glucoside, and quercetin-3-O-rhamnoside), catechin, and phenolic acids (gallic acid, protocatechulic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid) than flowers of subsequent developmental stages. The most significant differences were observed in the content of gallic acid; buds contained almost sixfold higher values than senescent flowers. Correlation analysis revealed a strong correlation between chromaticity parameters *a**, *b**, *a**/*b** ratio, *h*°, *L**, and major/total anthocyanins with values ranging from 0.60 to –0.84.**

The popularity and commercial marketability of rose flowers primarily depends on the size, coloration, longevity, and on the scent of rose flowers. A wide spectrum of colors can be attributed to various pigments such as anthocyanins, quercetins, and carotenoids (Eugster and Markifischer, 1991) in addition to other phenolics acting as copigments (Davies and Mazza, 1993).

During flower bud opening and senescence, various events take place in a well-defined sequence such as cell division, cellular differentiation, shifts in membrane permeability, cell elongation, and a wide range of gene expression (Kumar et al., 2008a) in association with changes in concentration of endogenous plant growth regulators and secondary metabolites (Kumar et al., 2008a; Mayak and Halevy, 1972; Sood and Nagar, 2003). Little information is available on phenolic content of developing rose petals. Sood and Nagar (2003) observed a sharp increase during flower development from the flower bud opening to full bloom in *Rosa bourboniana* Desport and *Rosa damascena* Mill. In other plants such as *Rosmarinus officinalis* L., the content of phenolic diterpenes and flavones increased during flower development. However, the level of phenolic acids remained constant (Del Baño et al., 2003).

Anthocyanin synthesis is an integral part of flower development and generally occurs at later stages of petal development (Weiss, 2000). The rapid petal growth, resulting from cell expansion, and anthocyanin accumulation in petals seem to be regulated by the same developmental signals (Weiss, 2000). The concentration of total anthocyanins in petals declined during flower development in *Rosa* ×*hybrida* 'Happiness' and 'Pink Coronet' (Ahuja et al., 1963), *Petunia* ×*hybrida* Hort. (Ferrante et al., 2006), and *Hydrangea macrophylla* Thunb. (Yoshida et al., 2008). This decline in phenolics and anthocyanins concentration at later stages of flower development may limit the role of the peroxidase/phenolics/ascorbic acid system in antioxidant defense and make the flower more vulnerable to oxidative stress (Takahama and Oniki, 1997). Plant tissue color is easily obtained with a portable colorimeter and several studies showed that there is a strong correlation between anthocyanins in flowers/leaves and one or more chromatic parameters (Katori et al., 2002; Schmitzer et al., 2009). Therefore, changes in the anthocyanin content could be linked to visual attributes and described by tristimulus values.

Potted miniature roses have become increasingly popular over the past few decades mostly as a result of the convenient small size, constant blooming, and rapid flower development, which also make it a suitable research material. In the present investigation, we have monitored the changes in concentration of various phenolic compounds and color parameters. To our knowledge, this work provides the first report on abundance of various anthocyanins, quercetins, catechin, and phenolic acids at four developmental stages of miniature rose 'KORcrisett'. The correlations between tristimulus color measurements and major/total anthocyanins were also evaluated.

Materials and Methods

Plant material and growth conditions. 'KORcrisett' rose plants were grown in a controlled environment glass greenhouse at 27/22 °C (day/night) equipped with a cooling system under natural photoperiod. The greenhouse environmental control system was set to start cooling at 27 °C. Relative humidity varied from 75% to 85%. Plants were irrigated daily using a flood irrigation system with a 4-min water (18 °C)

Received for publication 19 June 2009. Accepted for publication 17 July 2009. This work is part of program Horticulture No. P4-0013-0481 funded by the Slovenian Research Agency (ARRS).
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supply. Flower petals from the outermost whorls were colorimetrically analyzed and harvested in 15 replicates at four developmental stages (Fig. 1): bud [B (petals closed and fully pigmented with opened sepals)], partially open flower [PO (flowers with their two to three outer whorls unfurled)], fully open flower [FO (completely unfurled petals)], and senescent flower [S (petals rolled outward, discoloration detected, flower easily abscised)]. Petals from individual flowers were harvested at each stage and subjected to further analysis.

Flower color measurements. Flower color was measured by a portable colorimeter (CR-10 Chroma; Minolta, Osaka, Japan) with C illuminant. The colorimeter was calibrated with a white standard calibration plate before use. In the CIE L^* a^* b^* system of color representation, the L^* value corresponds to a dark–bright scale and represents the relative lightness of colors with a range from 0 to 100 (0 = black, 100 = white). The a^* and b^* values extend from –60 to 60; a^* negative is for green and a^* positive is for red, and b^* negative is for blue and positive for yellow. The a^*/b^* ratio was calculated for the purpose of correlation analysis with anthocyanic pigments. The hue angle ($h°$) is expressed in degrees from 0° to 360° (0° = red, 90° = yellow, 180° = green and 360° = blue). Color was measured in the middle of each petal (three replicates per flower) to ensure equal measurement conditions.

Extraction and high-performance liquid chromatograph determination of phenolic compounds. Petals of individual flowers were ground to a fine powder with liquid nitrogen and 2 g of powder extracted with 3 mL methanol containing 3% (v/v) HCOOH and 1% (w/v) 2,6-Di-*tert*-butyl-4-methylphenol (BHT) in an ultrasonic bath for 1 h. Treated samples were centrifuged for 7 min at 12,000 g_n. Supernatant was filtered through a polyamide filter (Chromafil AO-45/25; Macherey-Nagel, Düren, Germany) and transferred to a vial before injection in a high-performance liquid chromatograph (HPLC) system. Samples were analyzed using a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA) with a diode array detector at 280 nm (gallic acid, protocatechuic acid, catechin, chlorogenic acid, caffeic acid, *p*-coumaric acid), 350 nm (quercetins) and 530 nm (anthocyanins). A HPLC column (C18, 150 × 4.6 mm, Gemini 3µ; Phenomenex, Torrance, CA) protected with a Phenomenex security guard column operated at 25 °C was used. The injection volume was 20 µL and the flow rate maintained at 1 mL·min^{-1}. The elution solvents were aqueous 1% formic acid (A) and 99.8% acetonitrile (B). Samples were eluted according to the linear gradient described by Marks et al. (2007): 0 to 5 min, 3% to 9% B; 5 to 15 min, 9% to 16% B; 15 to 45 min, 16% to 50% B; 45 to 50 min, 50% isocratic; and finally washing and reconditioning of the column. The concentrations of phenolic compounds were assessed from peak areas and quantified with the use of corresponding external standards and anthocyanins by the use of a calibration curve of cyanidin-3,5-di-O-glucoside. Anthocyanins were further identified using a mass spectrometer (LCQ Deca XP MAX; Thermo Scientific) with an electroscopy interface 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 maintained at 1 mL·min^{-1}. Capillary temperature was 250 °C, the sheath gas and auxiliary gas were 20 and 8 units, respectively, the capillary voltage was 26 V, and spray voltage 4 V. Multipole Rf amplitude was 550 V_{p-p}. All compounds were expressed as µg·g^{-1} fresh weight (FW).

Fig. 1. Four stages of flower development: 1) bud; 2) partially open flower; 3) fully open flower; and 4) senescent flower.

Chemicals. The standards used to determine the phenolic compounds in samples were gallic acid, (+)-catechin, quercetin-3-O-rutinoside, cyanin chloride, kuromanin chloride, and chlorogenic acid from Sigma-Aldrich (Steinheim, Germany); catechin from Roth (Karlsruhe, Germany); protocatechulic acid from Merck (Darmstadt, Germany); and caffeic acid, *p*-coumaric acid, quercetin-3-glucoside, quercetin-3-O-rhamnoside, and peonidin-3-O-glucoside from Fluka (Buchs, Switzerland).

The chemicals for the sample preparation and mobile phases were methanol, BHT, and acetonitrile from Sigma-Aldrich and formic acid from Fluka. The water used in the mobile phase was bidistilled and purified with a Milli-Q water purification system by Millipore (Bedford, MA).

Statistical analysis. The results were analyzed using Statgraphics Plus 4.0 (Manugistics, Rockville, MD) program using one-way analysis of variance. Differences in phenolic concentrations among developmental stages were estimated with Duncan's multiple range test ($P < 0.05$). Multiple variable analysis with Pearson's product moment correlation coefficient (r) was calculated between anthocyanins and each of the color variables at $P < 0.05$.

Results

Petal color measurements. Color parameters a^*, b^*, and $h°$ declined at the successive stages of flower development. However, a^*/b^* ratio and lightness (L^*) were increased. The intense red of the buds changed to pink–violet in senescent flowers (Table 1). Analysis of the color parameters L^* and $h°$ revealed statistically significant differences among all flower developmental stages. An increase in the parameter L^* was observed with each change in flower stage; L^* increased for partially opened flowers 9.25%, fully opened flowers 15.60%, and senescent flowers 23.52% when compared with the bud stage. On the other hand, parameter $h°$ decreased with each change in flower stage; $h°$ was 10.91% lower for partially opened flowers, 22.43% lower for fully opened flowers, and as much as 48.11% lower for senescent flowers when compared with the bud stage.

Anthocyanin concentration during flower development. The HPLC chromatogram at 530 nm revealed different peaks corresponding to pelargonidin-3,5-di-O-glucoside > cyanidin-3,5-di-O-glucoside > pelargonidin-3-O-glucoside > peonidin-3-O-glucoside > cyanidin-3-O-glucoside. The concentration of the major anthocyanins (pelargonidin-3,5-di-O-glucoside and cyanidin-3,5-di-O-glucoside) in rose petals showed a significant negative trend during flower development. In partially opened flowers, the concentration of pelargonidin-3,5-di-O-glucoside was 27.32% lower than in buds and declined to 36.36% at senescent stage (Fig. 2A). Similarly, the concentration

Table 1. Chromatographic parameters (mean ± SE) of *Rosa* ×*hybrida* 'KORcrisett' petals at four flower stages.

Flower stage[z]	Chromatographic parameter (mean ± SE)				
	*a**	*b**	*a**/*b** ratio	*L**	*h*°
B	55.49 ± 1.66 b[y]	46.21 ± 3.60 c	1.40 ± 0.16 a	41.08 ± 0.72 a	29.33 ± 0.51 d
PO	55.94 ± 1.50 b	44.42 ± 2.69 c	1.44 ± 0.18 a	44.88 ± 0.69 b	26.13 ± 0.88 c
FO	51.45 ± 1.63 b	27.55 ± 1.39 b	2.06 ± 0.07 b	47.49 ± 1.01 c	22.75 ± 1.01 b
S	40.96 ± 1.88 a	11.30 ± 1.14 a	3.56 ± 0.26 c	50.74 ± 0.73 d	15.22 ± 1.11 a

[z]B = bud; PO = partially open flower; FO = fully open flower; S = senescent flower.
[y]Different letters (a, b, c, d) in rows denote statistically significant differences by Duncan's multiple range test at $P < 0.05$.

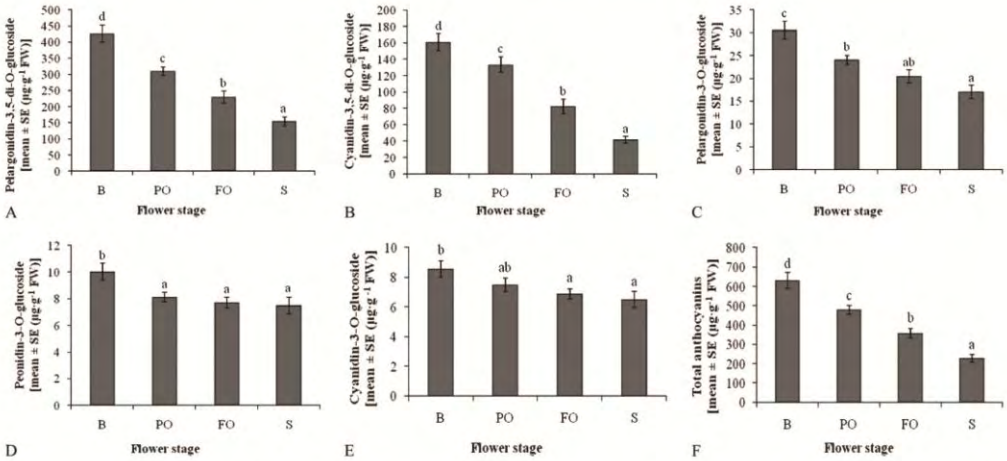

Fig. 2. Concentration of single (**A**–**E**) and total anthocyanins (**F**) in petals of *Rosa* ×*hybrida* 'KORcrisett' at four flower stages: bud (B), partially open flower (PO), fully open flower (FO), and senescent flower (S). Different letters (a–d) denote statistically significant differences by Duncan's multiple range test at $P < 0.05$.

of cyanidin-3,5-di-O-glucoside was low (26.07%) in senescent flowers in comparison with concentrations measured in buds (Fig. 2B). A similar trend was observed in the concentration of the minor anthocyanin, pelargonidin-3-O-glucoside (Fig. 2C) from bud (30.70 ± 1.84 μg·g^{-1} FW) to senescent stage (17.08 ± 1.48 μg·g^{-1} FW). The changes in the concentration of other minor anthocyanins (cyanidin-3-O-glucoside and peonidin-3-O-glucoside) during flower development were not consistent; however, statistically significant differences were observed between the bud stage and other flower stages (Fig. 2D–E). The concentration of total anthocyanins decreased from buds to senescent flowers and statistically significant differences were observed in the concentration of total anthocyanins among all developmental stages (Fig. 2F) with values ranging from 227.70 ± 20.47 μg·g^{-1} FW (senescent flower) to as much 630.68 ± 42.98 μg·g^{-1} FW (buds).

The changes in single/total anthocyanin concentration during flower development were not accompanied by a change in the ratio among three anthocyanins (pelargonidin > cyanidin > peonidin) except at the senescent stage in which a smaller relative concentration of cyanidin was detected as a result of an increase in the relative value of both pelargonidins and peonidin.

Correlation between anthocyanins and color measurements. Strong correlations were observed ($\alpha < 0.001$) among major/total anthocyanins, *a**, *b**, *a**/*b** ratio, *h*°, and *L** in rose petals (Table 2). The strongest correlation was obtained between *L** and cyanidin-3,5-di-O-glucoside (–0.84) followed by pelargonidin-3,5-di-O-glucoside (–0.83)

Table 2. Pearson's correlation coefficients between chromaticity parameters and major/total anthocyanins in *Rosa* ×*hybrida* 'KORcrisett' flowers.

Anthocyanin	Chromatographic parameter				
	*a**	*b**	*a**/*b* ratio	*h*°	*L**
Cyanidin-3,5-di-O-glucoside	0.82***[z]	0.70***	–0.60**	0.80***	–0.84***
Pelargonidin-3,5-di-O-glucoside	0.72***	0.75***	–0.75***	0.74***	–0.83***
Total anthocyanins	0.75***	0.74***	–0.65***	0.74***	–0.82***

[z]Pearson's correlation coefficients between chromaticity parameters and major/total anthocyanins with statistically significance of the estimated correlation presented at $^{**}P < 0.01$ and $^{***}P < 0.001$.

and total anthocyanins (–0.82). Similarly strong correlation coefficients were also observed among cyanidin-3,5-di-O-glucoside, a^* (0.82), and h° (0.80). Lowest correlation coefficients were obtained between a^*/b^* ratio, the concentration of cyanidin-3,5-di-O-glucoside (–0.60), and total anthocyanins (–0.65).

Quercetins and catechin during flower maturation. Quercetin-3-O-rhamnoside was the most abundant quercetin in rose petals followed by quercetin-3-O-glucoside and quercetin-3-O-rutinoside (Table 3). The concentration of quercetin-3-O-rhamnoside dropped significantly during flower development; in senescent flowers, the concentration was only 55.44% of the one in buds. A similar trend was observed for quercetin-3-O-rutinoside with statistically significant differences observed between bud stage and other flower developmental stages. Catechin was the dominant phenolic compound detected in rose petals and its concentration declined significantly during flower development. This decline was twofold from bud (4425.44 ± 271.52 $\mu g \cdot g^{-1}$ FW) to senescent stage (2040.90 ± 207.74 $\mu g \cdot g^{-1}$ FW).

Phenolic acids during flower maturation. Caffeic acid (Fig. 3D) and chlorogenic acid (Fig. 3C) were present in highest concentrations in rose petals followed by protocatechulic (Fig. 3B), *p*-coumaric (Fig. 3E), and gallic acid (Fig. 3A). The most significant differences were observed in the concentration of gallic acid; bud contained sixfold higher concentrations of gallic acid (27.66 ± 1.27 $\mu g \cdot g^{-1}$ FW) than senescent flowers (4.76 ± 0.62 $\mu g \cdot g^{-1}$ FW) and a clear negative trend was also observed from partially open (13.91 ± 1.15 $\mu g \cdot g^{-1}$ FW) to fully open flowers (7.47 ± 0.51 $\mu g \cdot g^{-1}$ FW). Similarly, the concentration of *p*-coumaric acid dropped from the stage bud to senescent flower by almost 80%. Significant differences were observed in the content of caffeic and chlorogenic acid at various stages of flower development. Buds contained more than twofold more caffeic acid than senescent flowers and threefold more chlorogenic acid.

Discussion

Flower development in *R.* ×*hybrida* 'KORcrisett' is accompanied by a substantial change in concentration of various phenolic compounds and petal color. As the flower developed, significant differences were observed in color parameters a^*, b^*, and L^*. The parameter a^* is associated with red coloration in rose petals (Biolley and Jay, 1993) and during 'KORcrisett' flower development, a steady decrease in this parameter was detected. Rose flowers in the final stages of flower development became paler and this characteristic visual change seems to be associated with a gradual increase of the parameter L^*. Many research studies have reported that the major anthocyanins in plant tissues exhibit a tight correlation with colorimetric indices such as lightness (L^*) and hue angle (h°) (Jia et al., 2008; Lancaster et al., 1997; Schmitzer et al., 2009). Correlation

Table 3. Concentration of quercetins and catechin in petals of *Rosa* ×*hybrida* 'KORcrisett' at four flower stages.

	Quercetins and catechin [mean ± SE ($\mu g \cdot g^{-1}$ fresh weight)]			
Flower stage[z]	Quercetin-3-O-glucoside	Quercetin-3-O-rutinoside	Quercetin-3-O-rhamnoside	Catechin
B	33.33 ± 1.97 NS[y]	11.75 ± 1.29 b	229.35 ± 13.12 c	4425.44 ± 271.52 c
PO	19.82 ± 0.84 NS	6.17 ± 0.47 a	135.75 ± 5.40 b	3673.41 ± 187.88 b
FO	16.13 ± 1.42 NS	6.57 ± 0.74 a	103.10 ± 10.00 a	2574.03 ± 182.59 a
S	24.24 ± 3.11 NS	8.63 ± 1.20 a	102.20 ± 13.19 a	2040.90 ± 207.74 a

[z]B = bud; PO = partially open flower; FO = fully open flower; S = senescent flower.
[y]Different letters in rows (a, b, c, NS = nonsignificant) denote statistically significant differences by Duncan's multiple range test at $P < 0.05$.

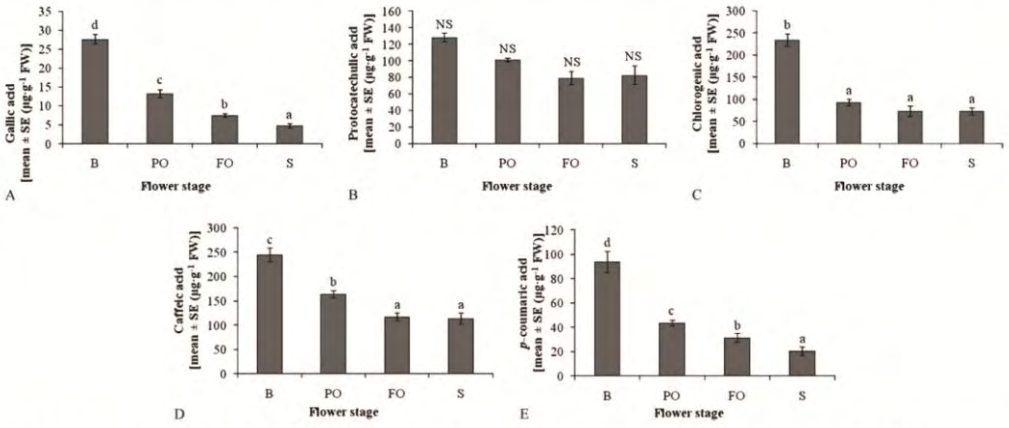

Fig. 3. Concentration of phenolic acids (**A**–**E**) in petals of *Rosa* ×*hybrida* 'KORcrisett' at four flower stages: bud (B), partially open flower (PO), fully open flower (FO), and senescent flower (S). Different letters (a–d, NS = nonsignificant) denote statistically significant differences by Duncan's multiple range test at $P < 0.05$.

analysis revealed a tight correlation between tristimulus values and major/total anthocyanins in 'KORcrisett' flower petals with the best correlation obtained between major/total anthocyanins and the parameter L^*. The correlation coefficient was negative between lightness (L^*) and the concentration of anthocyanins. Five anthocyanins detected in the petals of *R.* ×*hybrida* 'KORcrisett' were pelargonidin-3,5-di-O-glucoside, cyanidin-3,5-di-O-glucoside, pelargonidin-3-O-glucoside, peonidin-3-O-glucoside, and cyanidin-3-O-glucoside. Occurrence of similar anthocyanins was also reported in other rose cultivars (Asen, 1982; Biolley et al., 1994; Mikanagi et al., 1995). The major anthocyanins in miniature rose 'KORcrisett' throughout the development were pelargonidin-3,5-di-O-glucoside and cyanidin-3,5-di-O-glucoside, which is in accordance with the results of Biolley et al. (1994) who reported that diglucosides predominate over the related 3-monoglucosides and the co-occurrence of pelargonidin and cyanidin is common in modern roses (Mikanagi et al., 1995).

Although research reports on individual anthocyanin turnover in living plant tissues are scarce, it is well evident that the concentration of single and total anthocyanins is not constant during flower development (Dela et al., 2003). In our study, the concentration of three major anthocyanins (pelargonidin-3,5-di-O-glucoside, cyanidin-3,5-di-O-glucoside, pelargonidin-3-O-glucoside) dropped drastically from bud to senescent stage. However, the concentration of peonidin-3-O-glucoside and cyanidin-3-O-glucoside remained constant. Several factors might be responsible for this threefold decrease in the concentration of major anthocyanins from bud to senescent stage. First, active degradation of anthocyanin was observed in *Brunfelsia calycina* Benth. (Vaknin et al., 2005) and *P.* ×*hybrida* (Ferrante et al., 2006). Second, petal expansion mediated dilution of pigments and increase in flower weight (Vaknin et al., 2005). Third, it can be the result of a cessation of petal expansion at later stages of development. Probably anthocyanin concentration and the process of senescence are tightly linked phenomenon in rose flowers. Flower development in miniature rose 'KORcrisett' also revealed a significant negative trend in analyzed quercetins, catechin, and phenolic acids from bud to senescent stage. Quercetin-3-O-glucoside, quercetin-3-O-rhamnoside, and quercetin-3-O-rutinoside were the major quercetins in rose petals (Asen, 1982; Cai et al., 2005; Mikanagi et al., 1995) and their concentration declined with flower development. Gallic acid and protocatechuic acid, catechin, chlorogenic, caffeic, and *p*-coumaric acid (Cai et al., 2005; Kumar et al., 2008b; Velioglu and Mazza, 1991) are known components in rose petals and with progression of flower development in roses, the decrease in the concentration of these compounds was detected. Again, several factors might explain this gradation. Sequential biosynthesis of flavonols and anthocyanins in rose flowers could be strictly developmentally regulated like in *Eustoma grandiflora* (Raf.) Shinn. in which the flavonol concentration declined during floral development (Noda et al., 2004). On the other hand, it may be the result of a decline in PAL, CHI, DFR, and other gene transcriptions as was reported in *Malus* ×*domes*tica Borkh. flowers (Dong et al., 1998).

Flower development involves a number of interrelated processes such as growth, senescence, and abscission (Sood and Nagar, 2003) and our investigation showed a temporal decline in certain specific anthocyanic pigments and phenolics. This study provides an insight to explore the possible role of phenolics in regulation of flower development. The future work will evaluate the relative importance of these phenolics in a large number of rose cultivars for their antioxidant capacities and responses to pathogens at various stages of flower development.

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2.4 SPREMEMBE BARVE IN VSEBNOSTI FENOLOV MED RAZVOJEM CVETA PRI POKROVNIH VRTNICAH

SCHMITZER Valentina, VEBERIČ Robert, OSTERC Gregor in ŠTAMPAR Franci
Color and phenolic compound changes during flower development in groundcover rose
Journal of the American Society for Horticultural Science, 2010, 135: 195-202.
Prejeto: 19. 1. 2010, sprejeto: 15. 3. 2010

V študiji na rdeči sorti vrtnice 'KORcrisett' smo vizualne spremembe starajočih cvetov povezali z zmanjšano vsebnostjo antocianinov v venčnih listih. Zanimalo nas je, ali so podobne spremembe prisotne tudi pri razvoju cvetov belih in rožnatih sort pokrovnih vrtnic. Rezultate smo nadgradili s podatki o spremembi pH vrednosti celičnega soka in vsebnostjo vode ter težo cvetov od popka do senescentnega cveta.

Spremljali smo morfološke in kemične spremembe štirih razvojnih faz cvetov pri osmih sortah pokrovnih vrtnic: tri bele sorte ('NOAschnee', 'KOReb', 'Sea Foam'), dve rožnati sorti ('The Fairy', 'KORverladus') in tri rdeče sorte ('POUleas', 'MORedfar', 'Gärtnerfreude'). Barvne parametre smo izmerili s prenosnim kolorimetrom, posamezne fenole s pomočjo visokoločljivostne tekočinske kromatografije in skupne fenole spektrofotometrično. Pri vseh opazovanih sortah smo z razvojem cveta od popka do senescentnega cveta izmerili povečanje vrednosti barvnega parametra *L**, ki opisuje svetlost vzorca. Pri rdečih in tudi rožnatih sortah so se s staranjem cveta vrednosti parametra *a** močno zmanjšale. Kislost celičnega soka je bila pri vseh sortah največja v fazi popka, s staranjem cveta je pH vrednost naraščala. Sveža in suha teža petalov kot tudi vsebnost vode so se povečevali do faze popolnoma odprtega cveta in se v fazi senescentnega zmanjšali. V venčnih listih vseh preučevanih sort pokrovnih vrtnic smo potrdili prisotnost pelargonidin-3,5-di- glukozida in cianidin-3,5-di-glukozida ter številnih kvercetinov. Vsebnost skupnih antocianinov in skupnih kvercetinov je pri vseh sortah naraščala od faze popka do popolnoma odprtega cveta in zopet upadla v senescentnih cvetovih. Največje vrednosti skupnih fenolov pa so bile izmerjene s fazi popka in delno odprtega cveta. Tesne korelacije med barvnim parametrom *b** in pH vrednostjo celičnega soka ter med barvnim parametrom *a** in skupnimi antociani so bile izmerjene pri vseh preučevanih sortah pokrovnih vrtnic.

J. Amer. Soc. Hort. Sci. 135(3):195–202. 2010.

Color and Phenolic Content Changes during Flower Development in Groundcover Rose

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Additional index words. *Rosa* ×*hybrida*, cultivars, stages, colorimetric parameters, pH, phenolics

Abstract. **In the present study, the chemical and morphological status of eight cultivars of groundcover rose (*Rosa* ×*hybrida*) with a range of flower colors was investigated. From the methanolic extracts of rose petals collected from flowers at four developmental stages, several phenolic compounds were identified via high-performance liquid chromatography/mass spectrometry, including five anthocyanins, which are especially important for the visual attributes of rose flowers. Colorimetric parameters were also measured and correlated with total anthocyanins and cell sap pH levels. During flower development from bud to senescent stage, a significant trend was detected; lightness (*L**) increased, *b** decreased in all analyzed roses, and *a** decreased in pink and red cultivars. Cell sap pH level increased from bud to senescent petals; fresh weight, dry weight, and water content increased to fully open stage and were then reduced in senescent petals. Total anthocyanin and quercetin content increased from bud stage to fully open flowers, and was decreased in senescent ones. However, the highest content of total phenolics was measured in buds and partially opened flowers, respectively. Three distinct groups were formed according to the content of total anthocyanins and quercetins; white cultivars were most distant from the red ones, which were similar to the pink and light red cultivars.**

Groundcover rose is one of the most widely used roses in public spaces. Being relatively low maintenance, and having resistance to pests and diseases, winterhardiness, a long blooming period, and compact growth make them an ideal ornamental plant for parks as well as home gardens. A vast selection of different cultivars of *Rosa* ×*hybrida* with a color spectrum ranging from whites to intense purples is available.

Especially in red rose cultivars, a visible change in color during flower development and senescence is detected (Schmitzer et al., 2009). As the flower matures, different physiological, biochemical, and genetic processes occur in cells that contributes to the visual changes and characterizes a number of phases in flower life (Sood et al., 2006). In rose, it is common to observe several flower developmental stages on a single plant. Quite often, the intense color of rose buds changes to the subtle tones of fully opened flowers and the bluish hues of senescent petals. This phenomenon can be attributed to several factors: First, due to the changes in the content of various phenolic compounds and second, to an increase in cell sap pH level. Sood and Nagar (2003) and Sood et al. (2006) investigated the levels of acidic and bound phenols as well as total anthocyanins in rose petals and measured an increase in the initial stages of flower development, followed by a decrease at the fully open stage. However, in these studies on cut rose flowers, no data were reported on the content of phenolics in senescent rose petals. In contrast, a study of Barthe and Vaillant (1993) focused on postharvest changes in rose petal cell sap only from the partially open flower stage to late senescence, but no biochemical composition was determined. Thus, information about the relationship of individual anthocyanins to rose flower development stages is lacking.

Received for publication 19 Jan. 2010. Accepted for publication 15 Mar. 2010. This work is part of program Horticulture No. P4-0013-0481, funded by the Slovenian Research Agency (ARRS).
We would like to thank Arboretum Ljubljana for providing rose samples for the present study.
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The rose flower phenolic compounds, especially anthocyanic pigments, have been extensively studied, and considerable information about flower color biochemistry is available. Our past research on miniature rose flower development (Schmitzer et al., 2009) reported changes in individual phenolics at four developmental stages and correlated them to petal color modifications. However, only one cultivar was studied, and cellular changes in relation to phenolic composition during flower development were not monitored. The present work upgrades the insight into the biochemical composition of attached rose flowers during flower development from bud to full senescence with morphological and physiological changes in three different color groups of groundcover roses. Moreover, as the quantification of specific phenolics in groundcover cultivars of *R.* ×*hybrida* is still scarce, the new information provides a valuable insight to the phenolic composition of selected cultivars, which can be interesting for potential breeding material.

Materials and Methods

Plant material. Eight cultivarss of *R.* ×*hybrida* growing in Arboretum Ljubljana in Ljubljana, Slovenia (lat. 46.1°N, long. 14.6°E, 250 m altitude), were selected for this study and flowers were sampled in the beginning of Aug. 2009. Flower petals of three white flowering cultivars (NOAschnee, KOReb, and Sea Foam), two pink flowering cultivars (The Fairy and KORverlandus), and three red flowering cultivars (POUleas, MORedfar, and Gärtnerfreude) were collected at four different flower developmental stages (Fig. 1) as described earlier (Schmitzer et al., 2009): 1) bud [B (ends of sepals separated, petal color intense, petals still closed)], 2) partially open flower [POF (outer whorls unfurled, half open flower), 3) fully open flower [FOF (petals fully unfurled, full bloom)], 4) senescent flower [SF (petals rolled outward, discoloration detected, and easily abscised flower)].

Petals from individual flowers were hand plucked at each developmental stage, packed into paper bags, and transported

Fig. 1. Four stages of flower development in rose cultivar The Fairy: B = bud, POF = partially open flower, FOF = fully open flower, SF = senescent flower.

on ice to laboratory facilities, where they were immediately analyzed.

Petal color measurements. Flower color was measured by a portable colorimeter (CR-10 Chroma; Minolta, Osaka, Japan) with C illuminant. The colorimeter was calibrated with a white standard calibration plate before use. In CIE L^* a^* b^* system of color representation, the L^* value corresponds to a dark-bright scale and represents the relative lightness of colors with a range from 0 to 100 (0 = black, 100 = white). Color parameters a^* and b^* extend from –60 to 60; a^* negative is for green and a^* positive is for red and b^* negative is for blue and positive for yellow. The hue angle ($h°$) is expressed in degrees from 0 to 360, where 0° = red, 90° = yellow, 180° = green and 270° = blue. Color was measured in the middle of each petal (three replicates per flower; five flowers per stage) to ensure equal measurement conditions.

Fresh weight (FW), dry weight (DW), and water content determination. Fresh weight of petals was recorded immediately after harvest. Petal water content was determined as the percentage of total petal weight [(FW – DW)/FW × 100] by weighing samples of all petals from a single flower at four developmental stages. Petal DW was recorded after drying at 105 °C for 48 h in an electrical oven until constant weight was obtained. Five replicates per developmental stage were taken.

pH measurements. Relative changes in cell sap pH (correlating to changes in vacuolar pH) were determined by extracting 0.5 g of macerated petals in 5 mL of bidistilled water. After 2 h and occasional stirring, pH of the solution was measured with a pH meter (S20 SevenEasy™; Mettler Toledo, Columbus, OH). Each measurement was repeated on five individual flowers per developmental stage.

Extraction and high-performance liquid chromatography (HPLC) determination of phenolic compounds. Fresh petals of individual flowers were ground to a fine powder with liquid nitrogen and 0.5 g of powder was extracted with 2 mL of methanol containing 3% (v/v) formic acid and 1% (w/v) 2,6-Di-*tert*-butyl-4-methylphenol (BHT) in an ultrasonic bath for 1 h. Samples were centrifuged for 7 min at 12,000 g_n. Supernatant was filtered through Chromafil AO-45/25 polyamide filter (Macherey-Nagel, Düren, Germany) and was transferred to a vial before injection in a HPLC system. Samples were analyzed using a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA) with a diode array detector at 350 nm (quercetins) and 530 nm (anthocyanins). An HPLC column (150 × 4.6 mm, Gemini 3µ C18; Phenomenex, Torrance, CA), protected with a Phenomenex security guard column operated at 25 °C, was used. The injection volume was 20 µL and the flow rate was maintained at 1 mL·min^{-1}. The elution solvents were aqueous 1% formic acid (A) and 99.8% acetonitrile (B). Samples were eluted according to the linear gradient described by Marks et al. (2007): 0 to 5 min, 3% to 9% B; 5 to 15 min, 9% to 16% B; 15 to 45 min, 16% to 50% B; 45 to 50 min, 50% isocratic; and finally washing and reconditioning of the column. The concentrations of phenolic compounds were assessed from peak areas and were quantified with the use of corresponding external standards and anthocyanins by the use of a calibration curve of cyanidin-3,5-di-O-glucoside. Anthocyanins were further identified using a mass spectrometer (LCQ Deca XP MAX, Thermo Scientific) with an electroscopy interface (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 was maintained at 1 mL·min^{-1}. Capillary temperature was 250 °C, the sheath gas and auxiliary gas were 20 and 8 units respectively, the capillary voltage was 26 V, and the spray voltage was 4 V. Total anthocyanins and total quercetins were calculated as the sum of all identified anthocyanins and quercetins, respectively. All compounds were expressed as micrograms per gram DW.

Determination of total phenolic content. Extraction of petal samples for determining total phenolics was done as described above, but without the addition of BHT. The total phenolic content (TPC) was determined using the Folin-Ciocalteau phenol reagent method (Singleton and Rossi, 1965). To 100 µL of extract [diluted 1:8 (v/v) with MeOH], 6 mL of bidistilled water and 500 µL of Folin-Ciocalteau reagent were added; after resting between 8 s and 8 min at room temperature, 1.5 mL of sodium carbonate (20% w/v) was added. The extracts were mixed and left in an oven for 30 min at 40 °C before measuring absorbance on a spectrometer (ultraviolet/VIS Lambada Bio 20; Perkin Elmer, Waltham, MA) at 765 nm. A mixture of bidistilled water and reagent was used as blank. The TPC was expressed as gallic acid equivalents (GAE) milligrams per gram DW.

Chemicals. The standards used to determine the phenolic compounds in samples were quercetin-3-O-rutinoside and cyanin chloride from Sigma-Aldrich (Steinheim, Germany), and quercetin-3-glucoside, quercetin-3-xyloside, quercetin-3-arabinoside, quercetin-3-galactoside, and quercetin-3-rhamnoside from Fluka (Buchs, Switzerland). For the total phenolic content, Folin-Ciocalteu's reagent, sodium carbonate, gallic acid, and ethanol from Sigma were used. The chemicals for the sample preparation and mobile phases were methanol, BHT, and acetonitrile from Sigma-Aldrich and formic acid from Fluka. The water used in mobile phase was bidistilled and purified with a Milli-Q water purification system by Millipore (Bedford, MA).

Statistical analysis. The results were analyzed using the Statgraphics Plus 4.0 (Manugistics, Rockville, MD) program using one-way analysis of variance. Differences in phenolic concentrations among developmental stages were estimated with Duncan's multiple range test ($P < 0.05$). Multiple-variable analysis with Pearson's correlation coefficient (r) was calculated between color variable b^* and cell sap pH level and between color variable a^* and total anthocyanin content at $P < 0.05$. Multivariate statistical analysis (hierarchical cluster analysis, discriminate analysis, and classification) was conducted to interpret the differences in total anthocyanins and total quercetins among color groups.

Results and Discussion

Petal color measurements. White cultivars were all characterized by negative values for parameter a^* and higher

values for parameters b^* and h°, compared with pink and red cultivars (Table 1). Parameter a^* is associated with the amount of red coloration present in rose petals (Biolley and Jay, 1993), and our results show a clear increase from light pink cultivar The Fairy to intense red cultivars Gärtnerfreude and MORedfar. In pink and red cultivars, color parameter a^* declined at the successive stages of flower development, with statistically lowest values measured on senescent petals, except in cultivar MORedfar. This shift also corresponds well with the gradation in red color from buds to senescent flowers in red/pink cultivars. A postharvest study of Marousky and Carlyle (1985) on rose cultivar Better Times reported a decline in parameter a^* after 3 d of vase life, however, petals of the other two red cultivars studied did not show the same gradation in this color value. Similarly, in our study on white cultivars, no significant negative tendency in value a^* was detected, which can be explained by a low content of red pigments even at the initial phases of flower development. A steady decrease in value b^* was observed in all analyzed cultivars; the blue hues in the final stage of senescence became apparent, particularly in red cultivars. The petals faded to a bluish color, consistent with the reports of Itzhaki et al. (1990) on 'Mercedes' rose, where they visually assessed the color change in late senescence, but no colorimetric measurements were reported. The high degree of bluing in red rose petals held in water or preservative is in part due to the increase in pH caused by an increase in free ammonia. Higher pH levels cause structural modification in anthocyanic pigments, resulting in their blue color (Asen et al., 1971) expressed by a decrease of the b^* value. With the progression of flower development, lightness (L^*) was increased

Table 1. Colorimetric parameters of petals at four flower stages and eight cultivars of *Rosa ×hybrida*.

Cultivar	Flower color	Flower stage[z]	Colorimetric parameter (mean ± SE)			
			a^*	b^*	L^*	h°
NOAschnee	White	B	–10.16 ± 0.16 a[y]	20.34 ± 1.49 c	78.65 ± 1.22 a	113.9 ± 1.77 a
		POF	–7.65 ± 0.21 b	14.78 ± 0.35 b	84.41 ± 0.34 b	118.2 ± 1.19 b
		FOF	–8.09 ± 0.45 b	11.80 ± 0.71 a	84.22 ± 0.87 b	127.00 ± 10.4 c
		SF	–7.64 ± 0.26 b	11.03 ± 0.33 a	83.78 ± 0.59 b	125.20 ± 0.96 c
KOReb	White	B	–4.37 ± 0.36 c	45.14 ± 1.02 d	75.13 ± 0.68 a	96.21 ± 0.51 a
		POF	–3.31 ± 0.10 d	37.03 ± 0.97 c	77.69 ± 1.45 a	96.25 ± 0.30 a
		FOF	–7.65 ± 0.12 a	21.30 ± 0.80 b	81.27 ± 0.59 b	111.90 ± 1.55 b
		SF	–6.85 ± 0.06 b	10.01 ± 0.17 a	80.64 ± 1.06 b	124.40 ± 0.90 c
Sea Foam	White	B	–13.22 ± 0.87 a	29.12 ± 0.30 c	70.36 ± 0.61 a	106.90 ± 0.32 a
		POF	–6.25 ± 0.20 c	21.49 ± 0.82 b	81.51 ± 0.39 b	105.30 ± 0.82 a
		FOF	–6.07 ± 0.58 c	11.18 ± 0.27 a	84.24 ± 0.87 c	114.90 ± 0.73 b
		SF	–8.57 ± 0.17 b	12.08 ± 0.48 a	85.24 ± 0.32 c	125.40 ± 0.59 c
The Fairy	Light pink	B	13.13 ± 0.56 c	8.17 ± 0.31 c	58.37 ± 1.21 a	30.47 ± 0.57 a
		POF	12.94 ± 0.15 c	5.97 ± 0.20 b	65.87 ± 0.54 b	30.48 ± 1.70 a
		FOF	8.92 ± 0.18 b	5.29 ± 0.35 ab	73.99 ± 0.32 c	46.68 ± 0.32 c
		SF	4.84 ± 0.29 a	4.78 ± 0.40 a	76.04 ± 0.42 c	41.12 ± 1.00 b
KORverlandus	Dark pink	B	50.95 ± 0.78 c	6.67 ± 0.62 c	37.95 ± 0.68 a	7.57 ± 1.01 a
		POF	50.70 ± 0.22 c	–1.07 ± 0.42 b	50.80 ± 0.56 b	9.15 ± 0.64 a
		FOF	32.47 ± 0.90 b	–2.49 ± 0.30 a	60.92 ± 0.70 c	356.60 ± 1.61 b
		SF	27.02 ± 0.55 a	–1.47 ± 0.26 ab	64.21 ± 0.57 d	358.50 ± 0.38 b
POUleas	Light red	B	49.75 ± 0.28 c	3.54 ± 0.29 c	32.34 ± 1.05 a	3.75 ± 0.53 a
		POF	48.31 ± 0.20 bc	–3.63 ± 0.54 b	40.92 ± 0.77 b	353.00 ± 1.36 b
		FOF	46.63 ± 0.38 b	–3.34 ± 0.19 b	40.21 ± 0.49 b	357.50 ± 1.22 c
		SF	40.50 ± 1.64 a	–5.17 ± 0.59 a	44.66 ± 1.21 c	353.90 ± 1.22 b
MORedfar	Red	B	57.48 ± 0.69 b	14.09 ± 0.71 c	34.75 ± 0.33 a	13.36 ± 0.59 c
		POF	55.30 ± 1.42 ab	5.61 ± 0.13 b	42.19 ± 0.26 b	4.98 ± 0.27 b
		FOF	53.03 ± 1.11 a	2.99 ± 0.38 a	44.52 ± 0.29 c	2.68 ± 0.46 a
		SF	53.93 ± 0.86 a	2.10 ± 0.43 a	48.82 ± 0.37 d	4.16 ± 0.70 ab
Gärtnerfreude	Red	B	58.02 ± 2.01 c	5.87 ± 0.32 c	23.96 ± 0.35 a	4.35 ± 0.22 a
		POF	54.30 ± 0.94 bc	5.74 ± 0.50 bc	36.84 ± 0.63 b	7.77 ± 0.37 b
		FOF	52.00 ± 1.63 b	4.86 ± 0.27 b	38.80 ± 0.23 c	90.1 ± 0.12 b
		SF	43.58 ± 0.81 a	–1.34 ± 0.09 a	43.70 ± 0.52 d	358.40 ± 1.93 c

[z]B = bud, POF = partially open flower, FOF = fully open flower, SF = senescent flower.
[y]Different letters (a–d) in rows of individual cultivars denote statistically significant differences in colorimetric parameters by Duncan's multiple range test at $P < 0.05$ among flower developmental stages.

in all rose cultivars as flower petals visually became paler (Table 1). Similar changes in colorimetric parameters were described in a previous study on a red rose cultivar KORcrisett (Schmitzer et al., 2009) and cut 'Mercedes' rose flowers (Oren-Shamir et al., 2001) where the paler flower color, described with the parameter L^*, was also most evident. This could be due to an increase in water content and the dilution effect as well as lower content of specific pigments in some cultivars (Table 2, Figs. 3 and 5), respectively.

Fresh weight, dry weight, and water content. The mean FW, DW, and water content of petals in all eight cultivars generally increased from bud to fully open flower. The highest values of FW were measured at POF or FOF stage, and a decrease in FW was observed at the senescent stage of flower development in all cultivars except in 'MORedfar' (Fig. 2A). Similar results were reported for *Rosa damascena* and *Rosa bourboniana* flowers in the study of Sood et al. (2006), where FW of petals reached its maximum at fully open stage, but no measurements were taken at senescence. An increase in FW can be attributed to a high percentage of water content in enlarged cells of POF and FOF in most cultivars analyzed (Fig. 2D), suggesting that cell expansion and particularly water accumulation in vacuoles play an important role in the rose flower development. Petal DW increased from buds to partially or fully opened flowers and was again reduced in senescent flowers, except for cultivars KOReb and Sea Foam (Fig. 2B). Similarly, water content increased from bud to subsequent developmental stages, with the highest percentage measured in

Table 2. Anthocyanins in petals of eight cultivars of *Rosa ×hybrida* at four flower developmental stages.

Cultivar	Flower color	Flower stage[z]	Anthocyanin [mean ± SE (μg·g^{-1} DW)]		
			Pel-3,5-di-glu[y]	Cy-3,5-di-glu	Total anthocyanins
NOAschnee	White	B	4.93 ± 0.32 a[x]	0.10 ± 0.01 a	7.24 ± 0.04 a
		POF	20.46 ± 1.15 c	0.37 ± 0.05 ab	29.23 ± 1.15 c
		FOF	20.88 ± 1.27 c	0.67 ± 0.02 b	35.99 ± 1.53 d
		SF	12.28 ± 0.77 b	0.63 ± 0.11 b	19.55 ± 1.54 b
KOReb	White	B	4.49 ± 0.07 a	0.18 ± 0.02 a	5.56 ± 0.25 a
		POF	18.33 ± 0.41 c	0.46 ± 0.05 b	20.10 ± 0.34 d
		FOF	12.10 ± 1.29 b	0.43 ± 0.05 b	14.77 ± 1.38 c
		SF	7.47 ± 0.59 a	0.13 ± 0.02 a	8.96 ± 0.59 b
Sea Foam	White	B	10.26 ± 2.95 a	0.14 ± 0.06 a	12.71 ± 3.44 a
		POF	57.36 ± 6.58 c	0.53 ± 0.11 a	57.99 ± 7.33 c
		FOF	32.62 ± 3.20 b	2.09 ± 0.46 b	41.39 ± 3.18 bc
		SF	13.86 ± 0.59 a	0.74 ± 0.16 a	34.14 ± 1.70 b
The Fairy	Light pink	B	265.98 ± 22.07 a	4.81 ± 0.35 a	291.60 ± 21.33 a
		POF	475.00 ± 14.99 c	6.31 ± 0.18 c	500.41 ± 16.22 c
		FOF	374.86 ± 17.63 b	5.36 ± 0.18 b	397.32 ± 18.04 b
		SF	216.11 ± 11.70 a	2.36 ± 0.40 a	245.20 ± 14.31 a
KORverlandus	Dark pink	B	2,917.73 ± 117.79 c	388.97 ± 10.69 b	3,571.90 ± 152.36 c
		POF	2,688.87 ± 97.69 bc	458.12 ± 49.56 b	3,296.46 ± 125.41 bc
		FOF	2,481.84 ± 110.37 b	458.12 ± 49.56 b	3,106.54 ± 133.78 b
		SF	1,711.73 ± 110.13 a	220.87 ± 16.39 a	2,407.66 ± 118.26 a
POUleas	Light red	B	1,319.04 ± 94.95 ab	297.48 ± 18.52 ab	2150.86 ± 196.03 a
		POF	1,163.45 ± 56.17 a	249.26 ± 35.42 a	1824.34 ± 118.43 a
		FOF	1,839.56 ± 81.01 c	327.94 ± 25.85 b	3152.21 ± 293.94 b
		SF	1,434.97 ± 113.81 b	279.54 ± 12.88 ab	2944.42 ± 90.02 b
MORedfar	Red	B	4,071.74 ± 487.26 a	835.55 ± 25.75 a	5,049.05 ± 529.68 a
		POF	7,553.46 ± 224.71 c	1,350.40 ± 29.21 b	9,331.62 ± 311 09 c
		FOF	6,357.46 ± 399.37 b	1,395.82 ± 43.60 b	7,914.05 ± 464.02 bc
		SF	5,396.44 ± 148.41 b	807.22 ± 69.20 a	6,909.73 ± 414.32 b
Gärtnerfreude	Red	B	3,026.66 ± 907.54 a	586.94 ± 165.83 a	5,548.25 ± 505.90 a
		POF	7,199.89 ± 713.44 b	1,534.37 ± 141.51 b	9,115.76 ± 655.42 b
		FOF	9,374.99 ± 273.05 c	1,580.38 ± 82.10 b	11,434.30 ± 257.67 c
		SF	8,335.93 ± 330.60 bc	1,242.96 ± 73.44 b	10,233.70 ± 361.24 bc

[z]B = bud, POF = partially open flower, FOF = fully open flower, SF = senescent flower.
[y]Pel-3,5-di-glu = pelargonidin-3,5-di-O-glucoside, Cy-3,5-di-glu = cyanidin-3,5-di-O-glucoside.
[x]Different letters (a–d) in rows of individual cultivars denote statistically significant differences in individual and total anthocyanins by Duncan's multiple range test at $P < 0.05$ among flower developmental stages.

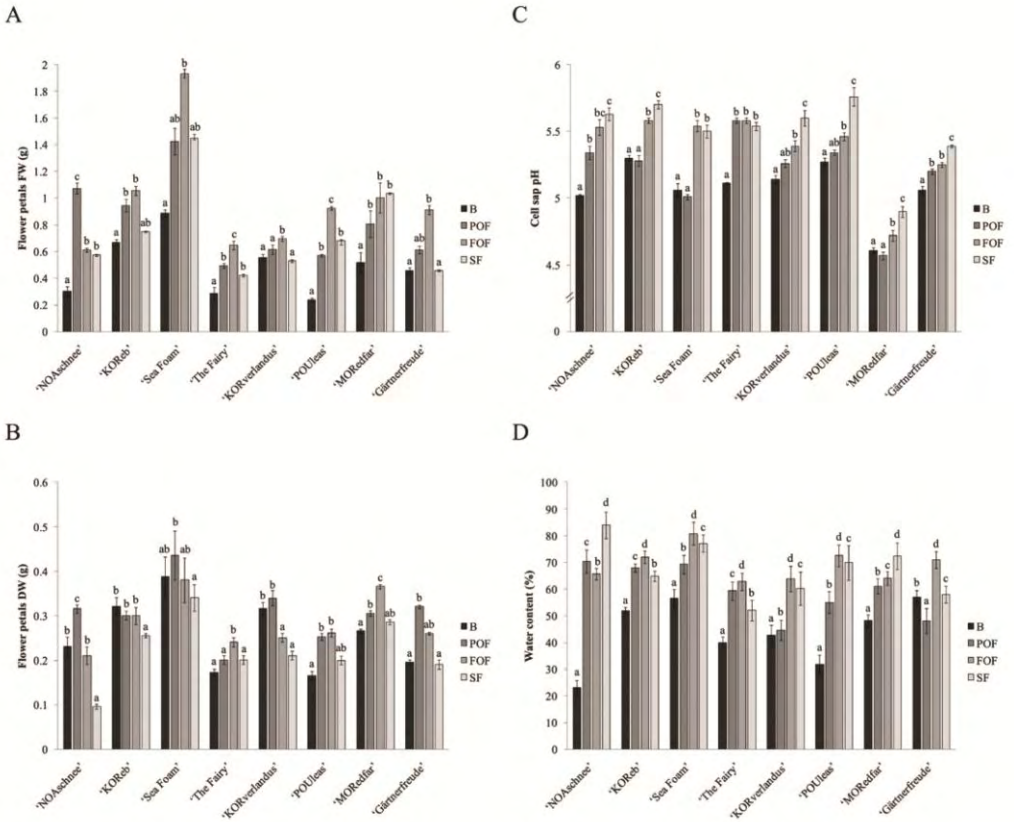

Fig. 2. Flower petal fresh weight (**A**), dry weight (**B**), cell sap pH level (**C**), and petal water content (**D**) at four flower stages (B = bud, POF = partially open flower, FOF = fully open flower, SF = senescent flower) and eight cultivars of *Rosa ×hybrida*. Different letters (a–d) denote statistically significant differences in parameters by Duncan's multiple range test at $P < 0.05$ among flower developmental stages of individual cultivars.

fully open flower stage and a slight drop in senescent petals (Fig. 2D) with the exception of 'NOAschnee', 'Gärtnerfreude', and 'MORedfar'. This is in accordance with the results of van Doorn and Schroder (1995) who state that petals of various rose cultivars were completely turgid or partially desiccated when shed. The relationship between relatively a high percentage of water in senescent petals and statistically lower values of fresh weight could be explained with two processes. The structural decomposition of the cell wall with a direct impact on cell wall yield was previously described in carnation petals (De Vetten and Huber, 1990). Also, in smaller part, it could be connected with a lower synthesis of secondary metabolites, and a higher breakdown and consequently a decrease in total phenolic content measured in the final stages of rose petal senescence (Fig. 3).

pH measurements. Generally, a weakly acidic vacuolar pH is most often maintained in plant cells and the slightest modifications often lead to changes in anthocyanin stability and, consequently, tissue color (Tanaka et al., 2005). In all analyzed cultivars, cell sap pH level of developing flowers increased from bud to senescent stage, respectively (Fig. 2C). Similarly, in cut 'Mercedes' and 'Sweet Promise' rose, cell sap pH increased with aging of petals during vase life (Barthe and Vaillant, 1993; Oren-Shamir et al., 2001). The high degree of postharvest bluing in rose petals can be linked to the increase in pH level (Asen et al., 1971). Aging petals of cut roses rapidly increase in ammonia, due to the proteolysis, resulting in an increase of cell sap pH, which is one of the physiological processes accompanying senescence (Kuiper et al., 1996).

The well-known bluing phenomenon in senescing red roses is tightly linked to it as studies describe a shift into the blue range of some anthocyanins when pH is increased. Asen et al. (1971) reported that cyanidin-3,5-di-O-glucoside was colorless at lower pH levels and formed complexes with quercetin derivates when pH was increased. These complexes can modify the color of the plant tissue to bluish hues. Although visually no blue coloration was apparent in senescent petals of white

cultivars, a similar increase of pH level was also measured in their cell sap. Interestingly, a tight correlation coefficient was calculated between the b^* value and cell sap pH values for all analyzed cultivars, including white ones (Table 3). Previous research on postharvest stages of rose reported similar correlation between cell sap pH and b^* value, however, only a single red rose cultivar was analyzed (Oren-Shamir et al., 2001).

Phenolic composition. As reported previously (Biolley et al., 1994; Mikanagi et al., 1995; Schmitzer et al., 2009), two predominant anthocyanic pigments are present in rose petals, namely pelargonidin-3,5-di-O-glucoside and cyanidin-3,5-di-O-glucoside (Table 2). Anthocyanins were detected in all analyzed roses, including white cultivars. Rose petals also contained small amounts of cyanidin-3-O-glucoside, pelargonidin-3-O-glucoside, and peonidin-3-O-glucoside (data not shown); however, the latter was not detected in white cultivars. Generally, during flower development, the content of total and major anthocyanins increased from bud to fully open flower and then dropped slightly at the senescent stage (Table 2). This characteristic trend was observed in most cultivars, although it was not always statistically significant and it seems that the mechanism of anthocyanin turnover in analyzed cultivars is fairly similar. However, Sood et al. (2006) spectrometrically evaluated total anthocyanin content in flowers of *R. damascena* and *R. bourboniana* and detected an increase in the first stages of flower development followed by a decrease in the half and fully opened flowers, with no data presented for senescent flower stage. Therefore, there is no universal trend of anthocyanin content during flower development and it seems that the level declines faster in some rose species than others. Anthocyanin degradation occurs in different plant organs due to a variety of environmental and developmental conditions; the latter can be linked to the vacuolar changes that decrease the stability of the pigments and cause chemical degradation or increased vulnerability to degrading enzymes (such as peroxidases and β-glucosidases) present in the vacuoles (Oren-Shamir, 2009). Increased pH level of the vacuolar sap in senescing petals of rose flowers (Fig. 2C) may have a negative effect on the stability of the anthocyanins and cause significant chemical degradation of the pigments. Moreover, as in the research on the fading color of petunia petals (Oren-Shamir, 2009), the altered vacuolar pH resulted in a modification of anthocyanin structure, which can also be linked to the fading and bluing of the senescent petals of red and pink rose cultivars.

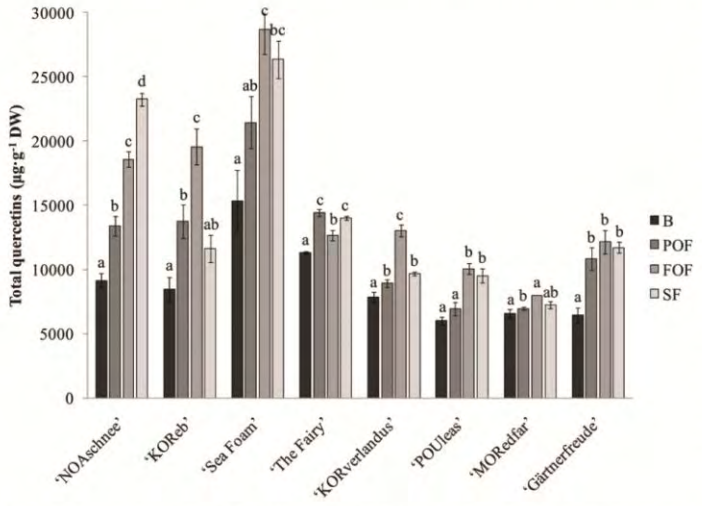

Fig. 3. Total quercetins in petals of eight cultivars of *Rosa ×hybrida* at four flower developmental stages (B = bud, POF = partially open flower, FOF = fully open flower, SF = senescent flower). Different letters (a–d) denote statistically significant differences in total quercetins by Duncan's multiple range test at $P < 0.05$ among flower developmental stages of individual cultivars.

Table 3. Pearson's correlation coefficients between different colorimetric parameters and cell sap pH level or total anthocyanins (fresh weight) for eight cultivars of *Rosa ×hybrida*.

Cultivar	b^*/cell sap pH	Correlation coefficient of a^*/total anthocyanins
NOAschnee	– 0.80***[z]	0.58*
KOReb	– 0.96***	0.77**
Sea Foam	– 0.79***	0.61*
The Fairy	– 0.71**	0.84***
KORvelandus	– 0.76**	0.83***
POUleas	– 0.73**	0.92***
MORedfar	– 0.76**	0.82***
Gärtnerfreude	– 0.89***	0.74*

[z]Pearson's correlation coefficients with statistical significance of the estimated correlations presented at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

A tight correlation was obtained between total anthocyanin content (FW) and color value a^* for all analyzed cultivars (Table 3), similar to the reports of Schmitzer et al. (2009), where only one red cultivar was studied. Although no apparent visual change in the coloration of white cultivars during flower development was detected, a correlation between anthocyanin content and color value a^* revealed a similar relationship to that of the red or pink cultivars analyzed. The content of cyanidin-3,5-di-O-glucoside can be related to blue coloration in red and pink cultivars at later stages of flower development when pH increases (Asen et al., 1971). However, in white cultivars, containing much lower amounts of cyanidin-3,5-di-O-glucoside, no blue hues were visually apparent, even in the late senescence stage. It seems that cyanidin-3,5-di-O-glucoside plays

an important role in color modification as the content of this pigment is as much as 1000-fold higher in red cultivars and they all exhibit petal bluing.

Six flavonols, belonging to the group of quercetins (Q), were identified in all cultivars, Q-3-rutinoside, Q-3-galactoside, Q-3-glucoside, Q-3-xyloside, Q-3-arabinoside, and Q-3-rhamnoside (data not presented), previously reported in several rose cultivars (Asen, 1982). The predominant Q-3-ramnoside and Q-3-glucoside comprised more than 90% of total quercetin content (TQC), respectively. TQC was always lowest in rose buds; a significant increase was detected in subsequent developmental stages. In 'NOAschnee', 'The Fairy', 'POUleas', and 'Gärtnerfreude', statistically highest TQC was measured in senescent petals (Fig. 3). Three distinct groups were formed when comparing the content of total anthocyanins and quercetins in eight cultivars (Fig. 4). White cultivars (NOAschnee, KOReb, and Sea Foam) fit in the first group, which was statistically most distant from the group of red cultivars (MORedfar and Gärtnerfreude). Two other pink and a light red cultivar (The Fairy, KORverlandus, and POUleas) were closer to the red color group.

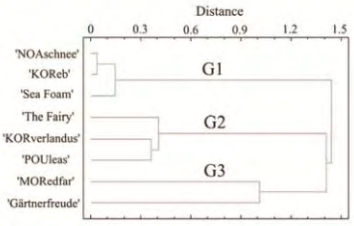

Fig. 4. Dendrogram of eight cultivars of *Rosa ×hybrida* using ward method based on square Euclidian distance from biochemical data (total anthocyanins and total quercetins). G1 to G3 represent different groups.

In comparison with other rose species, all analyzed cultivars of *R. ×hybrida* contained similar amounts of total phenolics (Fig. 5) on a dry weight basis. Kumar et al. (2009) reported TPC for *R. damascena* to be 145 mg·g^{-1} FW GAE, 178 mg·g^{-1} FW GAE for *R. bourboniana*, and as much as 254 mg·g^{-1} FW GAE for *Rosa brunonii*. However, Cai et al. (2005) report 189 mg·g^{-1} DW GAE for *Rosa chinensis*. The highest content of total phenolics was measured in buds or partially open flower stage, respectively. Phenolics play an important role in antioxidant defense by the peroxidase/phenolic/ascorbate system and the decline in these compounds make senescent flowers more vulnerable to oxidative stress (Takahama and Oniki, 1997). With the exception of 'Gärtnerfreude', all other cultivars had a low content of phenolics at the late senescence stage, suggesting that petals are more susceptible to oxidation leading to cell death in the final developmental stages.

According to Kumar et al. (2008), the flower is considered a strong sink of assimilates and the corolla continues to import dry matter throughout its development. But in the final stages of senescence, membrane destruction, electrolyte leakage, and a decrease in total protein (Sood et al., 2006) results in a decrease in petal dry and fresh weight. In the present study, the similarity of the developmental turnover in secondary metabolites among different cultivars of groundcover rose is established. The petal accumulates total and specific phenolics until full bloom stage, followed by a decrease in senescence. These mechanisms could be used in the selection of flower stages with highest values of secondary metabolites important for the pigment or confectionary industry. A detailed study on different rose species and cultivars is needed to describe these processes and further elucidate the individual phenolic turnover during flower development.

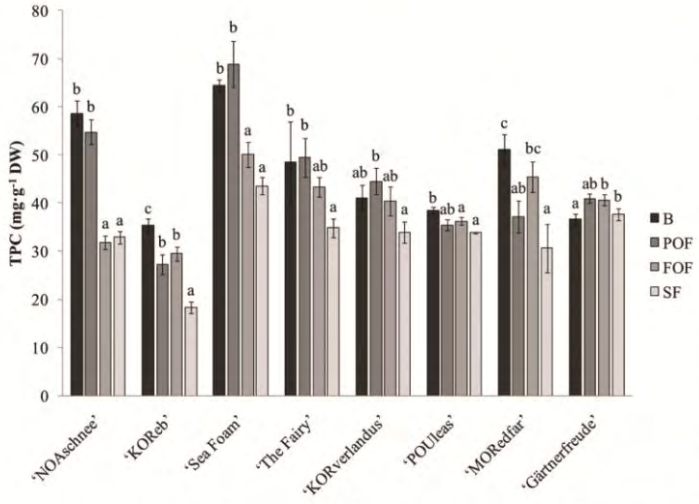

Fig. 5. Total phenolic content (TPC) in petals of eight cultivars of *Rosa ×hybrida* at four flower developmental stages (B = bud, POF = partially open flower, FOF = fully open flower, SF = senescent flower). Different letters (a–c) denote statistically significant differences in TPC by Duncan's multiple range test at $P < 0.05$ among flower developmental stages of individual cultivars.

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2.5 VPLIV PH SUBSTRATA NA VSEBNOST ANTOCIANINOV IN IZBRANIH FENOLNIH SNOVI PRI *Rosa* x *hybrida* 'KORcrisett'

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Substrate pH level effects on anthocyanins and selected phenolics in *Rosa* x *hybrida* 'KORcrisett'
Acta Agriculturae Slovenica, 2010, 95: 5-11.
Prejeto: 6. 10. 2009, sprejeto: 24. 2. 2010

Vsebnost fenolnih snovi v rastlinskih celicah je v veliki meri odvisna od okoljskih razmer kot so temperatura, vodni status, vsebnost mikro- in makrohranil, prisotnost patogenov in drugih stresorjev. Kislost substratne mešanice močno vpliva na dostopnost hranil in s tem na prehranjenost in status rastline.

Preučevali smo vpliv pH vrednosti substrata na količino antocianov, kvercetinov, katehina in fenolnih kislin v venčnih listih vrtnice *Rosa* x *hybrida* 'KORcrisett' in določili tri obravnavanja: pH 3,3 (kisel substrat), pH 4,7 (kontrola) in pH 7,3 (bazičen substrat). Obenem smo spremljali vpliv kislosti substratne mešanice na tvorbo cvetov. Fenolni profil smo določili s pomočjo tekočinske visokoločljivostne kromatografije v povezavi z masnim spektrofotometrom. Rastline, posajene v substrat s pH vrednostjo 4,7 so pri drugem vzorčenju v povprečju razvile 40 % več cvetov v primerjavi s prvim vzorčenjem, vrtnice v drugih dveh obravnavanjih pa 5,9 % (pH vrednost 3.3) in 8,1 % (pH vrednost 7,3) manj. Vendar pa so venčni listi rastlin, sajenih v substratno mešanico pH vrednosti 4,7, vsebovali najmanjše vsebnosti antocianov, kvercetinov, katehina in fenolnih kislin. V primerjavi s prvim vzorčenjem je se vsebnost prevladujočih (pelargonidin-3,5-di-glukozida in cianidin-3,5-di-glukozida) in skupnih antocianov v petalih vrtnic statistično značilno povečala pri rastlinah, ki so bile sajene v substratni mešanici s pH vrednostma 3,3 in 7,3. Vsebnost skupnih antocianov je bila tako kar 60,9 % in 66,7 % večja v venčnih listih rastlin, sajenih v substratni mešanici pH vrednosti 7,3 in 3,3 v primerjavi s tistimi, sajenimi v substrat s pH vrednostjo 4,7. Vsebnost kvercetinov je bila v drugem vzorčenju prav tako večja v petalih rastlin, sajenih v mešanici pH vrednosti 3,3 in 7,3. Vsebnost fenolnih kislin je bila pri drugem vzorčenju statistično značilno manjša pri vseh obravnavanjih, največje razlike pa so bile izmerjene v venčnih listih rastlin, sajenih v substratno mešanico pH vrednosti 4,7.

DOI: 10.2478/v10014-010-0001-5

COBISS Code 1.01

Agrovoc descriptors: rosa, anthocyanins, phenolic compounds, aromatic compounds, soil ph, soil chemicophysical properties, flowers, chemical composition, proximate composition, soil fertility

Agris category code: F60, P35

Substrate pH level effects on anthocyanins and selected phenolics in *Rosa* × *hybrida* L. 'KORcrisett'

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Received October 6, 2009; accepted February 24, 2010.
Delo je prispelo 6. oktobra 2009; sprejeto 24. februarja, 2010.

ABSTRACT

The effect of substrate pH level (4.7, 3.3 and 7.3) on the anthocyanin, quercetin compounds, catechin and phenolic acids concentrations in petals of *Rosa* × *hybrida* L. 'KORcrisett' and on the number of flowers per plant was investigated. The phenolic profiles of this plant were established for the first time by the use of HPLC/MS. Plants potted in a substrate with pH 4.7 developed significantly more flowers compared to those planted in an acidic (3.3) and alkaline (7.3) pH levels. However, the concentration of anthocyanins, quercetin compounds, catechin and phenolic acids was always lowest in the petals of 'KORcrisett' rose plants potted in pH level 4.7. Compared to the first sampling, a significant increase in the concentration of major and total anthocyanins and quercetin compounds was measured in the petals of plants potted in pH level 3.3 and 7.3, but not in the plants potted in pH level 4.7, respectfully.

Key words: rose, pH, substrate, anthocyanins, phenolic compounds

IZVLEČEK

VPLIV pH SUBSTRATA NA ANTOCIANE IN FENOLE PRI *Rosa* × *hybrida* L. 'KORcrisett'

Preučevali smo vpliv pH substrata (4,7, 3,3 in 7,3) na koncentracije antocianov, kvercetinov, katehina in fenolnih kislin v petalih *Rosa* × *hybrida* L. 'KORcrisett' ter spremljali število cvetov na posamezno rastlino. Sestava in koncentracija fenolnih spojin je bila pri tej rastlini prvič določena s pomočjo HPLC/MS tehnike. Rastline, ki so bile posajene v substrat s pH 4,7, so razvile statistično značilno več cvetov, kot rastline, posajene v kisel (3,3) oziroma baličen (7,3) pH, vendar pa so bile koncentracije antocianov, kvercetinov, katehina in fenolnih kislin v pH 4,7 najnižje. V primerjavi s prvim vzorčenjem, je koncentracija prevladujočih in skupnih antocianov v petalih močno narasla pri rastlinah, ki so bile posajene v substrat s pH 3,3 in 7,3. Podobnega trenda nismo opazili pri rastlinah, posajenih v pH 4,7.

Ključne besede: vrtnica, pH, substrat, antociani, fenolne spojine

1 INTRODUCTION

Roses are one of the most important, diverse and widely planted ornamentals with over 150 species and more than 20.000 cultivars (Cai et al., 2005) with color specter ranging from subtle whites, yellows and pinks to intense purple, orange and red tones. The color of various plant tissues, such as flower petals (Mikanagi et al., 1995) and leaves (Schmitzer et al., 2009a), can be attributed to anthocyanins and other phenolics, for example quercetins, acting as copigments (Eugster and Markifischer, 1991).

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Flower pigments accumulate in the epidermal cell vacuoles (Hughes et al., 2007) and their concentration, intensity and hue depends on both internal, such as microenvironment conditions in the vacuoles and developmental stage (Schmitzer et al., 2009b), and external factors. Among the latter light (Cominelli et al., 2007), temperature (Dela et al., 2003), nutrient deficiency (Juszczuk et al., 2004) and substrate pH (Smith et al., 2004a) has been reported to alter anthocyanin and carotenoid concentration in various plant tissues. Root substrate pH also affects nutrient solubility (Smith et al., 2004b; Papafotiou et al., 2007) and influences root formation (Harbage et al., 1998) with a direct impact on overall status of the plant. In roses, root growth was inhibited when plants were exposed to either pH 8 or pH 4 in comparison with plants grown in pH 6. Additionally, plant growth, leaf size and chlorophyll levels were not affected in plants at pH 4, while all these variables were reduced at pH 8 in comparison with plants grown at pH 6 (Zieslin and Snir, 1989).

We hypothetisized that the concentration of phenolic compounds (anthocyanins, quercetin compounds, catechin and phenolic acids) in petals of the miniature rose 'KORcrisett' differs according to substrate pH levels. The objective of our study was thus to determine the effects of the substrate pH level on the concentration of secondary metabolites in rose petals, important from the commercial aspect of miniature rose production as well as from the viewpoint of plants response to external stress. As plants grown outside their acceptable pH range show signs of chlorosis and general decline (Smith et al., 2004 a) a reduction in the number of flowers can also be expected in miniature roses potted in acidic or alkaline pH levels.

2 MATERIALS AND METHODS

2.1 Plant material and growth conditions

Rosa × *hybrida* L. 'KORcrisett' plants were planted in 1.3 l plastic pots (14 cm in diameter), containing a growth medium, prepared by mixing 80% of black peat and 20% of mineral component (sand). The substrate pH levels were modified by liming with calcium carbonate; the amount of lime was calculated on the base of a pH curve and three different pH levels were set up; treatment A with pH level 3.3, treatment B with pH level 4.7 and treatment C with pH level 7.3. For each treatment 15 plants were planted; the experiment was a randomized block design on a single bench. Plants were grown from the beginning of August to September 2008 in a controlled environment glass greenhouse at 27/22 °C (day/night) equipped with a cooling system under natural photoperiod. The greenhouse environmental control system was set to start cooling at 27 °C. Relative humidity ranged from 75-85 %. Plants were irrigated daily, using a flood irrigation system with 4 minutes water (18 °C) supply. The number of flowers per plant (buds to senescent flowers) was counted at the beginning of the experiment on 12. Aug. 2008 (day 0) and on 8. Sept. (day 28). At the same time petals (flower developmental stage 3; Muller et al., 1998) for the extraction of phenolics were collected and immediately frozen in liquid nitrogen and stored at -18 °C prior to further analysis.

2.2 Extraction and determination of phenolic compounds

For the analysis of phenolic compounds (anthocyanins, quercetin compounds and selected phenolics), frozen petals were ground to a fine powder with liquid nitrogen. A sample of 2 g was extracted with 3 mL methanol containing 3% (v/v) HCOOH and 1% (w/v) 2,6-di-*tert*-butyl-4-methylphenol (BHT) in an ultrasonic bath for one hour. After extraction, the treated samples were centrifuged for 7 min at 12,000 g_n. The supernatant was filtered through Chromafil AO-45/25 polyamide filter (Macherey-Nagel, Düren, Germany) and transferred to a vial prior to injection into the high-performance liquid chromatography (HPLC) system. The samples were analyzed using a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA) with a diode array detector at 280 nm (gallic acid, protocatechuic acid, catechin, *p*-coumaric acid), 350 nm (quercetins) and 530 nm (anthocyanins). A Phenomenex (Torrance, CA) HPLC column C18 (150 mm x 4.6 mm, Gemini 3µ) protected with a Phenomenex Security guard column, operated at 25 °C, was used. The injection volume was 20 µL and the flow rate was 1mL min^{-1}. The elution solvents were aqueous 1% formic acid (A) and acetonitrile (B). The samples were eluted according to the linear gradient described by Marks et al. (2007): 0–5min, 3–9% B; 5–15 min, 9–16% B; 15–45min, 16–50% B; 45–50min, 50% isocratic; and finally washing and reconditioning of the column. The concentrations of selected phenolic compounds were assessed from peak areas and quantified with the use of corresponding external standards and anthocyanins by the use of calibration curve of cyanidin-3,5-di-O-glucoside. Anthocyanins were further identified using a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX, San Jose, USA) with an electroscopy interface (ESI) operating in positive ion mode from *m/z* 115 to 800. The injection volume was 10 µL and the flow rate maintained at 1mL min^{-1}. Capillary temperature was 250 °C, the sheath gas and auxiliary gas were 20 and 8 units respectively, the capillary voltage was 26 V and spray voltage 4 V. Multipole Rf amplitude was 550 V_{p-p}. All compounds were expressed as µg g^{-1} FW.

2.3 Chemicals

The standards used to determine the phenolic compounds in samples were gallic acid, (+)-catechin, quercetin-3-O-rutinoside, cyanidin-3,5-di-O-glucoside and cyanidin-3-O-glucoside from Sigma-Aldrich (Steinheim, Germany), catechin from Roth (Karlsruhe, Germany), protocatechulic acid from Merck (Darmdstadt, Germany), caffeic acid, *p*-coumaric acid, quercetin-3-O-glucoside, quercetin-3-O-rhamnoside and peonidin-3-O-glucoside from Fluka (Buchs, Switzerland). The chemicals for the sample preparation and

mobile phases were methanol, BHT and acetonitrile from Sigma-Aldrich and formic acid from Fluka. The water used in mobile phase was bidistilled and purified with a Milli-Q water purification system by Millipore (Bedford, MA).

2.4 Statistical analysis

Statistical analysis was conducted with the program Statgraphics Plus 4.0 (Statgraphics, Herndon, VA). One-way analysis of variance ANOVA was used for analysis of the effect of substrate pH level on the number of flowers per plant and concentration of anthocyanins, quercetins and selected phenolics in rose petals. Differences in phenolic concentrations among pH treatments were estimated with Duncan's multiple range test ($P < 0.05$).

3 RESULTS

3.1 The number of flowers

On the first sampling, miniature rose plants on average produced 9.25 flowers per plant with no significant differences observed among pH treatments. However, after 28 days, the number of flowers per plant was significantly affected by substrate pH level (Fig.1). Compared to the first sampling, a decline in the number of flowers per plant was detected, when 'KORcrisett' rose was potted in both alkaline (8.10% less flowers per plant) and acidic pH levels (5.88% less flowers per plant) in contrast to pH 4.7, where plants developed 40% more flowers, respectfully.

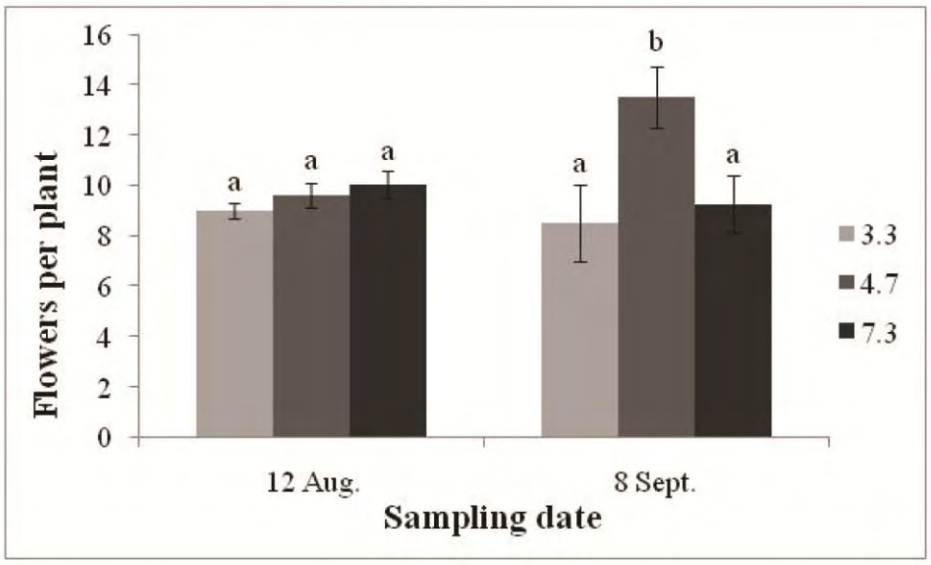

Figure 1: The effect of substrate pH on the number of flowers per plant at the beginning of the experiment (12 Aug.) and on day 28 (8 Sept.). Values carrying the same letters (a, b) for each set of dates do not differ significantly by Duncan's multiple range test at $P < 0.05$.

3.2 Anthocyanins

The HPLC chromatogram revealed five peaks at 530 nm, corresponding to two pelargonidin-based, two cyanidin-based and one peonidin-based glucoside. On the first sampling, flower petals averagely contained 283.79 µg g^{-1} FW pelargonidin-3,5-di-O-glucoside, 67.55 µg g^{-1} FW cyanidin-3,5-di-O-glucoside, 20.94 µg g^{-1} FW pelargonidin-3-O-glucoside, 10.36 µg g^{-1} FW cyanidin-3-O-glucoside, 9.87 µg g^{-1} FW peonidin-3-O-glucoside and 380.80 µg g^{-1} FW total anthocyanins, with

no statistical differences observed among pH treatments (Table 1). After 28 days, significant differences among pH treatments were detected in the concentrations of all anthocyanins with the lowest values obtained from plants potted in 4.7 pH level. The concentration of two major anthocyanins (pelargonidin-3,5-di-O-glucoside and cyanidin-3,5-di-O-glucoside) was 64.20% and 70.01% higher in the acidic pH level and 67.31% to 60.12% higher in alkaline pH level when compared to 4.7 pH level. However, the greatest difference was observed in the concentration of pelargonidin-3-O-glucoside; in both acidic and alkaline pH levels a more than two fold increase was measured when compared to pH level 4.7. Total anthocyanins were 60.88% and 66.73% higher in alkaline and acidic pH levels compared to pH level 4.7 on the second sampling. Generally, no statistical differences in single and total anthocyanins were detected between acidic and alkaline pH levels, except in the concentrations of cyanindin-3-O-glucoside and peonidin-3-O-glucoside, where higher concentrations were detected in alkaline pH levels. Interestingly, the concentration of cyanidin-3,5-di-O-glucoside in petals of plants potted in acidic and alkaline pH level on the second sampling was more than threefold higher than on the first and consequently, the concentration of total anthocyanins in these treatments also increased 66.73% to 60.88%. The concentration of the major anthocyanin (pelargonidin-3,5-di-O-glucoside) and three minor ones, however, were similar to those measured on the first sampling.

Table 1: Effect of substrate pH level on the concentration of anthocyanins (µg g^{-1} FW) in petals of *Rosa* × *hybrida* L. 'KORcrisett' on two sampling dates.

Sampling date	pH Level	Anthocyanin[1] [mean ± SE (µg g^{-1})] Pel-di-glu	Cy-di-glu	Pel-glu	Cy-glu	Peo-glu	Total anthocyanins
12 Aug.	3.3	267.12 ± 16.40 a[2]	69.91 ± 6.00 a	20.33 ± 1.47 a	10.85 ± 0.78 a	10.10 ± 0.84 a	375.57 ± 24.30 a
	4.7	265.17 ± 18.93 a	62.06 ± 6.00 a	19.35 ± 1.48 a	9.65 ± 0.70 a	8.75 ± 0.71 a	357.55 ± 25.53 a
	7.3	319.08 ± 21.51 a	70.68 ± 7.34 a	23.15 ± 1.57 a	10.57 ± 0.68 a	10.75 ± 0.86 a	409.28 ± 35.56 a
8 Sept.	3.3	324.40 ± 29.65 b	268.33 ± 22.39 b	27.32 ± 3.64 b	10.24 ± 1.67 ab	9.54 ± 1.62 ab	636.04 ± 52.42 b
	4.7	197.56 ± 13.22 a	157.83 ± 13.72 a	13.57 ± 0.66 a	6.19 ± 1.40 a	6.28 ± 0.24 a	381.48 ± 27.75 a
	7.3	330.53 ± 34.82 b	253.19 ± 31.60 b	27.74 ± 4.02 b	13.65 ± 2.34 b	12.23 ± 1.60 b	613.74 ± 69.50 b

[1] Anthocyanin: Pel-di-glu, Pelargonidin-3,5-di-O-glucoside; Cy-di-glu, Cyanidin-3,5-di-O-glucoside; Pel-glu, Pelargonidin-3-O-glucoside; Cy-glu, Cyanidin-3-O-glucoside; Peo-glu, Peonidin-3-O glucoside.

[2] Values carrying the same letters (a-b) for each set of dates do not differ significantly by Duncan's multiple range test at $P < 0.05$.

3.3 Quercetin compounds and catechin

Three quercetin compounds were determined in the petals of *Rosa* × *hybrida* L. 'KORcrisett', the predominant quercetin-3-O-rhamnoside and two minor ones (quercetin-3-O-glucoside and quercetin-3-O-rutinoside). On the first sampling petals on average contained 70.87 µg g^{-1} FW quercetin-3-O-rhamnoside, 21.81 µg g^{-1} FW quercetin-3-O-glucoside and 7.33 µg g^{-1} FW quercetin-3-O-rutinoside, with no statistical differences observed among pH treatments (Table 2). On the second sampling, however, the lowest values of major and minor quercetin compounds were obtained from petals of plants, potted in a 4.7 ph level. Statistically significant differences in the concentration of the two most abundant quercetin compounds were also detected between acidic and alkaline pH levels; the concentration of quercetin-3-O-rhamnoside was from 98.31% to 55.77% higher and quercetin-3-O-glucoside from 123.37% to 70.41% higher than in pH level 4.7. Compared to the first sampling, the concentration of all quercetin compounds in petals was higher on the second sampling in both acidic and alkaline pH levels but lower in 4.7 pH level. The concentration of the predominant quercetin compound increased 61.72% and 59.28% in acidic and alkaline pH treatments and decreased by 25.95% in pH level 4.7 and a similar trend was detected for quercetin-3-O-rutinoside. The average concentration of catechin in petals of rose 'KORcrisett' on the first sampling was 2006.66 µg g^{-1} FW and after 28 days, the concentration only increased in the petals of plants potted in an acidic pH level. Compared to the first sampling the concentration was 20.50% higher in pH level 3.3 and from 8.05% to 14.51% lower in pH levels 4.7 and 7.3.

Table 2: Effect of substrate pH level on the concentration of quercetin compounds and catechin (µg g^{-1} FW) in petals of *Rosa* × *hybrida* L. 'KORcrisett' on two sampling dates.

		Quercetin compounds[1] and catechin [mean ± SE (µg g^{-1})]			
Sampling date	pH Level	Q-rhamn	Q-glu	Q-rut	Catechin
12 Aug.	3.3	75.28 ± 6.65 a[2]	19.64 ± 1.23 a	6.32 ± 0.57 a	1936.52 ± 103.86 a
	4.7	77.30 ± 7.14 a	23.42 ± 1.54 a	7.64 ± 0.60 a	1973.64 ± 72.32 a
	7.3	60.04 ± 6.54 a	22.38 ± 1.62 a	8.02 ± 0.54 a	2109.81 ± 82.28 a
8 Sept.	3.3	121.74 ± 9.45 c	30.20 ± 1.54 c	13.89 ± 1.52 b	2333.64 ± 179.42 b
	4.7	61.39 ± 3.94 a	13.52 ± 0.86 a	6.08 ± 0.82 a	1826.65 ± 105.03 a
	7.3	95.63 ± 7.65 b	23.04 ± 2.32 b	12.76 ± 1.27 b	1842.47 ± 148.71 a

[1] Quercetin compounds: Q-rhamn, Quercetin-3-O-rhamnoside; Q-glu, Quercetin-3-O-glucoside; Q-rut, Quercetin-3-O-rutinoside.

[2] Values carrying the same letters (a, b,c) for each set of dates do not differ significantly by Duncan's multiple range test at $P < 0.05$.

3.4 Phenolic acids

Four phenolic acids were extracted from the petals of miniature rose 'KORcrisett': gallic acid, protocatechulic acid, caffeic acid and *p*-coumaric acid (Table 3). On the first sampling rose petals averagely contained 31.37 µg g^{-1} FW gallic acid, 121.65 µg g^{-1} FW protocatechulic acid, 133.56 µg g^{-1} FW caffeic acid and 52.57 µg g^{-1} FW *p*-coumaric acid. After 28 days, the lowest concentrations of all phenolic acids were detected in the petals of plants potted in pH 4.7 and the highest in pH 3.3. The concentration of gallic acid was considerably lower on the second sampling; the decrease was more than seven fold in pH level 4.7, four fold in pH level 7.3 and more than two fold in pH level 3.3. Similarly, the concentrations of protocatechulic acid, caffeic acid and *p*-coumaric acid were significantly lower on the second sampling in both pH levels 4.7 and 7.3 and remained constant or even increased in the acidic pH level.

Table 3: Effect of substrate pH level on the concentration of phenolic acids (µg g^{-1} FW) in petals of *Rosa* × *hybrida* L. 'KORcrisett' on two sampling dates.

		Phenolic acid [mean ± SE (µg g^{-1})]			
Sampling date	pH Level	Gallic acid	Protocatehulic acid	Caffeic acid	*p*-Coumaric acid
12 Aug.	3.3	27.24 ± 2.02 a[1]	104.42 ± 9.11 a	123.34 ± 6.51 a	48.04 ± 3.60 a
	4.7	33.04 ± 2.30 a	131.18 ± 11.72 a	137.63 ± 7.52 a	52.57 ± 3.81 a
	7.3	33.82 ± 2.17 a	129.34 ± 10.08 a	139.70 ± 4.28 a	57.09 ± 3.42 a
8 Sept.	3.3	11.28 ± 2.03 b	104.62 ± 15.07 b	118.79 ± 16.0 b	66.43 ± 7.90 b
	4.7	4.39 ± 0.64 a	59.83 ± 3.78 a	81.29 ± 5.62 a	35.82 ± 3.91 a
	7.3	7.82 ± 1.17 ab	69.01 ± 9.90 a	82.27 ± 10.78 a	46.36 ± 4.41 a

[1] Values carrying the same letters (a-b) for each set of dates do not differ significantly by Duncan's multiple range test at $P < 0.05$.

4 DISCUSSION

Overall, pH of the substrate had a significant effect on the number of flowers per plant as well as on the phenolic concentration in rose petals, as it affects nutrient uptake into plants (Smith et al., 2004a, Papafotiou et al., 2007) and consequently, reproductive efficiency. Similarly, the production of flowers in *Senecio vulgaris* L. was fewer in plants, grown in a nutrient deficient substrate (Brown and Molyneux, 1996) and acidic and alkaline pH levels caused significant changes in flowering of tobacco plants (Pasqua et al., 1991). Miniature rose plants developed significantly less flowers when grown in a substrate with pH levels 3.3 and 7.3 compared to pH level 4.7. As early as 1930, a lower substrate pH level (an average of 5.7 is mentioned as optimal) was reported to have a positive effect on the growth of different rose cultivars (Zieslin and Snir, 1989). A clear increase in flower production was also noted when rose plants were grown in a peat and Lelite substrate amended with ammonium. Yields per ft^2 of roses increased as the proportion of NH_4^+ to NO_3^- increased causing a decrease in pH of the rhizosphere (White ad Richter, 1973; Findenegg et al., 1986).

In contrast, the concentration of major and minor anthocyanins in rose petals increased in more acidic and alkaline pH levels. External stressors, such as substrate pH level, promote anthocyanin synthesis as was demonstrated by Hawrylak-Nowak (2008) who reported an increase in anthocyanin concentration dependant on substrate alkalinity in maize (*Zea mays* L.). Pelargonidin-3,5-di-O-glucoside and cyanidin-3,5-di-O-glucoside were the prevailing anthocyanic pigments in 'KORcrisett' petals, which is in accordance with the results of Biolley et al. (1994) and Mikanagi et al. (1995) who obtained similar results in other rose cultivars. Similarly to the research of Mikanagi et al. (1995) *Rosa* × *hybrida* L. 'KORcrisett' petals contained three minor anthocyanic pigments: pelargonidin-3-O-glucoside, cyanidin-3-O-glucoside and peonidin-3-O-glucoside, all significantly affected by substrate pH level. Quercetin-3-O-glucoside, quercetin-3-O-rhamnoside and quercetin-3-O-rutinoside are the major quercetin compounds in rose flowers (Mikanagi et al., 1995; Cai et al., 2005) and, like anthocyanins, their concentration was lowest in flowers of the plants, potted in 4.7 pH level. Among the phenolic acids gallic, protocatechuic acid, caffeic acid and *p*-coumaric acid were previously reported by Cai et al. (2005) and Kumar et al. (2008) in other rose cultivars and were significantly affected by pH level. According to our research, the optimal pH level of the substrate for increased flowering of miniature rose 'KORcrisett' was 4.7, however when an increase in phenolic concentration is preferential a modified pH level could be used to produce plants with flowers, which contain more anthocyanins and other phenolic compounds.

5 ACKNOWLEDGEMENT

This work is part of program Horticulture No. P4-0013-0481, funded by the Slovenian Research Agency (ARRS).

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3 RAZPRAVA IN SKLEPI

3.1 RAZPRAVA

V prvem sklopu vzorčenj in poskusov na okrasnih rastlinah smo pri sedmih sortah *Acer palmatum* Thunb. ugotavljali količino izbranih sekundarnih metabolitov v jesensko obarvanih listih in s prenosnim kolorimetrom merili barvne parametre. Poleg določitve genotipskih razlik vsebnosti antocianov pri pahljačastih javorjih nas je zanimalo, ali položaj lista na drevesu (topofiza) vpliva na kopičenje preučevanih spojin. Glede na razlike v položaju vej na drevesu smo določili tri obravnavanja: (1) veja z vrha krošnje, (2) srednje ležeča veja in (3) veja na spodnjem delu provodnika. Zanimale so nas tudi povezave med vsebnostjo prevladujočih antocianinov v listih ter izbranimi barvnimi parametri.

V poskus smo vključili sedem sort pahljačastih javorjev; dve celoletno rdeči sorti *Acer palmatum* 'Bloodgood' in *A.p.* 'Coreanum' ter pet sort, ki se jeseni intenzivno rdeče obarvajo: osnovno vrsto *Acer palmatum* Thunb., *A. p.* var *palmatum*, *A. p.* 'Semi K2', *A. p.* 'Dissectum rubrifolium' in *A. p.* 'Dissectum nigrum'. Liste smo analizirali jeseni 2007, ko so ti na vzorčenih drevesih začeli dobivati značilno jesensko barvo. Jesenska obarvanost listov na analiziranih drevesih je bila postopna, kot opisano pri drevesnih vrstah *Rhus*, *Euonymus* in *Acer* (Ishikura, 1976).

Barvo smo spremljali kolorimetrično z določitvijo CIE LAB parametrov. Pričakovali smo, da bosta genotip in položaj lista na drevesu močno vplivala na intenzivnost rdeče obarvanosti in svetlost površine listov. Ker je obarvanost listja pri drevesih v veliki meri odvisna od osvetlitve in količine fotosintetsko aktivnega sevanja (Hughes in sod., 2007), smo vzorčili le liste z oboda krošnje in enotne južne orientiranosti dreves.

Ugotovili smo, da je vrednost barvnega parametra *a**, ki se giblje med -60 (zeleno) in 60 (rdeče), močno odvisna od sorte pahljačastega javorja kot tudi od položaja lista na drevesu (Schmitzer in sod., 2009a). Največjo vrednost tega parametra smo izmerili na površini listov z vrha krošnje osnovne vrste *Acer palmatum*, najmanjšo pa na površini listov iz spodnje veje *A. p.* var. *palmatum.* Praviloma je bil parameter *a** pri analiziranih sortah največji v (1) obravnavanju in najmanjši v (3) obravnavanju. Položaj je torej statistično značilno vplival na vrednost parametra, ki opisuje intenzivnost rdeče obarvanosti v jesenskem listju pahljačastih javorjev, saj so bili listi iz vrha krošnje vedno bolj rdeči od tistih, ki so rastli v spodnjem delu drevesa krošnje. Razlike v vrednosti parametra *a** so bile statistično značilne tudi pri merjenju obarvanosti površine listov dveh celoletno rdečih sort javorjev, kar dodatno potrjuje povezanost intenzivnosti rdeče barve s položajem lista na drevesu in topofizo. Zanimiva je tudi ugotovitev, da so listi celoletno rdeče obarvanih sort 'Coreanum' in 'Bloodgood' statistično značilno manj intenzivno rdeče obarvani v primerjavi z osnovno vrsto pahljačastega javorja v jesenskem času.

Sorta in pozicija na drevesu sta vplivali tudi na CIE faktor *L**, ki predstavlja svetlost površine lista in se giblje od 0 (črno) do 100 (belo). Temnejša obarvanost površine listov pahljačastih javorjev je neposredno povezana z intenzivnejšo rdečo barvo, ki je v jesenskem času bolj temna od zelene. Pri preučevanih sortah so bile statistično značilne najvišje vrednosti tega parametra izmerjene na listih iz spodnjih vej, najtemneje pa so bili obarvani listi z vrha krošnje. Najmanjše razlike pri spremljanju pozicijskega učinka na parameter *L** so se pokazale pri merjenju površine celoletno rdečih sort pahljačastih javorjev, največje pa pri osnovni vrsti *Acer palmatum.*

Vrednost barvnega parametra *b**, ki določa odtenke od modre (-60) do rumene (60), je bila pri analiziranih sortah največja v (3) obravnavanju. Količina rumene obarvanosti je tako po pričakovanju največja v listih z vrha krošnje, razlike pa so bile ugotovljene med sortami. Tudi *a**/ *b** razmerje je bilo močno odvisno od položaja lista na drevesu; največje vrednosti so bile izmerjene v listih z vrha krošnje.

Da bi kolorimetrične parametre, ki določajo barvo jesenskega listja pri pahljačstih javorjih, povezali z vsebnostjo pigmentov, smo vzorce analizirali s pomočjo tekočinske visokoločljivostne kromatografije. V vseh sedmih sortah smo potrdili prisotnost dveh antocianinov, prevladujočega cianidin-3-glukozida in manj zastopanega cianidin-3-rutinozida (Schmitzer in sod., 2009b). Prav tako smo ugotovili prisotnost številnih antocianinov, derivatov cianidina in delfinidina, vendar v bistveno manjših količinah. Podobne rezultate o vsebnosti antocianinov v listih javorjev v svojih raziskavah podajajo tudi Ji in sod. (1992) in Fossen in Andersen (1999) ter Hughes in sod., (2007) v listih celoletno rdečih okrasnih sort lesnatih rastlin. Rezultati raziskave so obenem skladni s prejšnjimi dognanji o vsebnosti antocianinov v listih kritosemenk, saj je cianidin-3-glukozid dokazano prevladujoč antocianin (Harborne, 1976; Ishikura, 1976), nekoliko manj pogosti pa so drugi derivati cianidina in delfinidina (Fossen in Anderssen, 1999). Vsebnost derivatov delfinidina do sedaj v listih pahljačastih javorjev ni bila potrjena.

Največje vrednosti prevladujočega antocianina smo izmerili v listih iz vrha krošnje *A. p.* var *palmatum*, najmanjše pa v listih vej spodnjega dela debla pri isti sorti in ugotovili kar 52-kratno razliko v vsebnosti cianidin-3-glukozida med položajema (Schmitzer in sod., 2009b). Vsebnost cianidin-3-glukozida je bila vedno največja v listih, vzorčenih na vejah z vrha krošnje in najmanjša v listih, ki so izraščali iz spodnjih vej. Tudi pri sortah, ki imajo celoletno rdeče obarvane liste, je bil opaženo enako zmanjšanje kar dodatno potrjuje vpliv topofize na vsebnost prevladujočega antocianina v listih pahljačastih javorjev. Razlika v vsebnosti cianidin-3-glukozida v listih z vrha in spodnjega dela drevesa je bila pri sorti 'Bloodgood' namreč kar štirikratna, pri sorti 'Coreanum' pa dvakratna. Zato vsebnost cianidin-3-glukozida lahko predstavlja količinski biokemijski parameter določen tudi s položajem lista na drevesu. Vsebnost cianidin-3-rutinozida je bila po drugi strani bistveno manjša in pri večini sort največja v listih iz vrha krošnje, vendar zmanjšanje ni bilo tako očitno kot pri prevladujočem antocianinu.

Pri analiziranih sortah pahljačastega javorja so bile potrjene tesne korelacije med parametrom *a** in vsebnostjo cianidin-3-glukozida. Korelacijski koeficienti so bili največji pri dveh celoletno rdečih sortah 'Bloodgood' in 'Coreanum', manjši pa pri sortah, pri katerih se je listje postopno rdeče obarvalo jeseni. Rdeča barva listov javorjev je močno odvisna od vsebnosti antocianinov v vakuolah celic epiderma in gobastega mezofila (Hughes in sod., 2007). Barvna koordinata *a** s svojo višjo vrednostjo torej do določene mere predstavlja količino antocianinskih (»rdečih«) pigmentov v tkivu, zato je tesna povezava med tem parametrom in vsebnostjo prevladujočega cianidin-3-glukozida v listih pahljačastih javorjev pričakovana.

Izmerjene so bile korelacije med vsebnostjo prevladujočega antocianina in kolorimetričnimi parametri *b**, *h*° (kot med 0 in 360°, ki določa barvni odtenek) in *L** (Schmitzer in sod., 2009a). Vsebnost cianidin-3-glukozida je bila s temi parametri obratno sorazmerno povezana, pozitivna korelacija pa je bila izmerjena tudi med vsebnostjo cianidin-3-glukozida in razmerjem *a**/*b**. Povezave med manj zastopanim cianidin-3-rutinozidom in izmerjenimi barvnimi parametri niso bile statistično značilne pri vseh analiziranih sortah. Izražanje rdeče barve pri pahljačastih javorjih je zato močno povezano z vsebnostjo prevladujočega antociana v listih, manj pa z vsebnostjo drugih izmerjenih rdečih pigmentov.

Dosedanje raziskave poročajo o zvezi med antociannii in izmerjenimi barvnimi parametri na plodovih sadnih rastlin (Iglesias in sod., 2008) in zelenjadnic (Lancaster in sod., 1997), Katori in sod. (2002) pa so opisali povezave v venčnih listih več sort lotusa. Spremembe barve jesenskega listja okrasnih rastlin pa razen v naši študiji niso bile opisane in opredeljene s CIE LAB parametri ter njihovo povezavo z vsebnostjo rdečih pigmentov. Na izražanje barve vplivajo tudi drugi dejavniki kot so vsebnost nekaterih fenolnih snovi in kopigmentov, pH vrednost celičnega soka, prisotnost kovinskih ionov v raztopini in fiziološke ter morfološke značilnosti celic in tkiv (Cooper-Driver, 2001). Zato korelacije med vsebnostjo prevladujočih antocianov in barvnimi parametri vedno predstavljajo le del vpliva na vizualno dojemanje barve.

Slika 2: Vizualne spremembe barve javorja *Acer palmatum* (Thunb.) z veje krošnje (levo), sredinske pozicije (sredina) in veje spodnjega dela drevnine (desno)
Figure 2: Visual changes of autumn color of maple *Acer palmatum* (Thunb.) leaves from the terminal branch (left), middle branch (middle) and base branch (right)

V drugem sklopu poskusov na okrasnih rastlinah smo preučevali vpliv parametrov, ki vplivajo na barvo in vsebnost izbranih sekundarnih metabolitov pri vrtnicah. V prvi poskus smo vključili miniaturno vrtnico *Rosa* x *hybrida* 'KORcrisett', v drugem vzorčenju pa razširili meritve na osem sort pokrovnih vrtnic. Vizualna sprememba barve venčnih listov rdečih in rožnatih sort vrtnic, ki je povezana s staranjem cveta, je bila do sedaj namreč slabo opisana, še zlasti v zadnjih razvojnih fazah cveta. Da bi natančno ovrednotili spremembo, smo barvo izmerili s prenosnim kolorimetrom in jo opisali s pomočjo CIE LAB parametrov. Obenem smo s pomočjo visokoločljivostne tekočinske kromatografije v venčnih listih določili prevladujoče antocianine, kvercetine, katehin in fenolne kisline. Določili smo štiri obravnavanja: (1) popek, (2) delno odprt cvet, (3) popolnoma odprt cvet, in (4) senescenten cvet. Izmerjene barvne vrednosti, vsebnosti sekundarnih metabolitov in druge parametre smo povezali s korelacijsko analizo.

Intenzivna rdeča barva venčnih listov vrtnice 'KORcriset' s staranjem cveta vizualno nekoliko zbledi, v zadnji fazi senescentnega cveta pa prehaja v modrikasto-vijoličaste odtenke. Očitno je torej, da je barva cvetov pri vrtnicah poleg vsebnosti pigmentov v vakuolah pogojena tudi s starostjo venčnih listov. Vendar pa je bila izmerjena vrednost barvnega parametra *a**, ki določa vsebnost rdeče barve, statistično značilno najnižja le v (4) obravnavanju, razlik med prvimi tremi fazami razvoja cveta pa ni bilo moč izmeriti (Schmitzer in sod., 2009c). Verjetno je večja svetlost venčnih listov razvijajočih se cvetov vrtnice 'KORcrisett' povezana tudi z učinkom razredčitve celičnega soka in večje vsebnosti vode v celicah in ne le z zmanjšanjem rdeče obarvanosti. Obenem pa so se značilne razlike namreč pokazale v vrednosti barvnega parametra *L** (svetlost), ki se je z razvojem in staranjem cveta povečala. Statistično značilno je na isti rastlini najtemnejši popek, delno odprt in popolnoma odprt cvet sta svetlejša, na površini venčnih listov senescentnih cvetov pa smo izmerili največje vrednosti parametra *L**.

Razlike so bile statistično značilne tudi pri vrednotenju parametra *b**, ki je bil največji v venčnih listih popkov in delno odprtih cvetov, najmanjši pa pri senenscentnih cvetovih. Manjše vrednosti CIE LAB parametra *b** nakazujejo prehod v modrikaste tone v zadnji fazi razvoja cveta pri rdeči sorti vrtnice 'KORcrisett', kar je bilo tudi vizualno jasno opazno. Vrednost parametra *h*°* je bila negativno povezana z razvojem cveta; statistično najmanjše vrednosti so bile izmerjene v senescentnih cvetovih. Ta parameter je moč opredeliti z manjšo nasičenostjo (saturacijo) barvnega odtenka, kar je povezano tudi z bledenjem barve in s tem večjim izmerjenim parameter *L**. Z razvojem cveta se je močno spremenilo tudi razmerje *a**/*b**, ki je bilo statistično značilno namanjše pri venčnih listih popkov in delno odprtih cvetov, največje pa pri senenscentnih cvetovih vrtnice 'KORcrisett'. Slednje je bilo visoko predvsem zaradi prehoda barve v vijoličaste odtenke in sorazmerno visokih vrednosti rdeče obarvanosti, ki smo jo izmerili v zadnji fazi razvoja cveta.

V vseh razvojnih fazah cvetov pri vrtnici 'KORcrisett' smo potrdili prisotnost petih antocianinov: dveh prevladujočih pelargonidin-3,5-di-glukozida in cianidin-3,5-di-glukozida ter manj zastopanih pelargonidin-3-glukozida, cianidin-3-glukozida in peonidin-3-glukozida. Podobno poročajo tudi Biolley in sod. (1994) ter Kumar in sod. (2008), ki so v venčnih listih rdečih sort vrtnic analizirali vsebnosti pelargonidina, cianidina in

peonidina. V novejših požlahtnjenih sortah pa, podobno kot kažejo ugotovitve naše raziskave, prevladujejo diglukozidi pelargonidina in cianidina (Mikanagi in sod., 1995).

Vsebnost posameznih in skupnih antocianinov v venčnih listih vrtnice 'KORcrisett' se je z razvojem cveta statistično značilno zmanjšala (Schmitzer in sod., 2009c). Največje vsebnosti pelargonidin-3,5-diglukozida so bile izmerjene v popkih, v senescentnih cvetovih pa je bila vsebnost za 36 % manjša. Podobni rezultati so bili izmerjeni tudi s primerjavo vsebnosti cianidin-3,5-diglukozida in pelargonidin-3-glukozida v popkih in senescentnih cvetovih, kjer so bile očitne razlike med posameznimi razvojnimi fazami cveta (popek > delno odprt cvet > popolnoma odprt cvet > senescenten cvet). Vsebnosti drugih, manj zastopanih antocianinov so bile sicer največje v popkih in najmanjše v senescentnih cvetovih, vendar pa med drugimi razvojnimi fazami ni bilo zaznati statistično značilnih razlik. Vsebnost skupnih antocianinov se je gibala med 228 μg g^{-1} sveže mase v senescentnih cvetovih do 630 μg g^{-1} sveže mase v popkih vrtnice 'KORcrisett'. Razmerje vsebnosti posameznih antocianinov je bilo v razvojnih fazah podobno, le v fazi senescentnega cveta se je vsebnost cianidina v primerjavi s pelargonidinom in peonidinom zmanjšala.

Na zmanjšanje vsebnosti antocianinov v venčnih listih razvijajočih cvetov vrtnice vpliva več procesov, ki so bili do sedaj raziskani v študijah na okrasnih rastlinah. V petalih tropske vrste *Brunfelsia calycina* Benth. so Vaknin in sod. (2005) potrdili aktiven razpad antocianinskih pigmentov, podobno pa poročajo Ferrante in sod. (2006), ki so spremljali zmanjšanje vsebnosti antocianinov pri petuniji (*Petunia* x *hybrida* L.). Poleg tega je zmanjšanje vsebnosti antocianinov od faze popka do popolnoma odprtega in senescentnega cveta moč deloma pripisati učinku razredčitve (dilucije pigmentov) in povečanju vsebnosti vode ter vakuole v celicah (Vaknin in sod., 2005). Podatke o vsebnosti pigmentov, preračunane na svežo maso venčnih listov vrtnice 'KORcrisett', smo zato v razširjeni študiji na osmih sortah pokrovnih vrtnic nadgradili z rezultati, ki upoštevajo vsebnost vode v celicah in obenem spremenjeno pH vrednost vakuolnega soka s staranjem cveta, ki prav tako vpliva na vsebnost analiziranih antocianinov.

V venčnih listih vrtnice 'KORcrisett' smo med fenolnimi snovmi analizirali tudi vsebnosti treh kvercetinov: kvercetin-3-ramnozida, kvercetin-3-glukozida in kvercetin-3-rutinozida – najpogosteje zastopanih kvercetinov v vrtnicah kot poročajo Mikanagi in sod. (1995) in Cai in sod. (2005). Z razvojem cveta se je vsebnost prevladujočega kvercetin-3-ramnozida močno zmanjšala; v senescentnih cvetovih je bila za 55 % manjša od izmerjene vsebnosti v popkih (Schmitzer in sod., 2009c). Podobno zmanjšanje je bilo dokazano tudi v vsebnosti kvercetin-3- rutinozida, ki je bila največja v venčnih listih popkov, in katehina, ki je bil v venčnih listih vrtnice 'KORcrisett' tudi najbolj zastopan med analiziranimi fenolnimi snovmi. Vsebnost slednjega je bila v senescentnih cvetovih dvakrat manjša kot v popkih.

Med fenolnimi kislinami sta v venčnih listih vrtnice 'KORcrisett' prevladovali kavina in klorogenska kislina. Poleg prevladujočih fenolnih kislin smo potrdili prisotnost galne, protokatehulne in *p*-kumarne kisline, kar je skladno z rezultati, ki jih navajajo Cai in sod. (2005) in Kumar in sod. (2008). Vsebnosti kavne in klorogenske kisline so bile največje v venčnih listih popkov, podobno kot vsebnosti drugih fenolnih kislin, ki smo jih analizirali v petalih. Statistično značilno največje razlike so bile prisotne, ko smo primerjali vsebnosti

galne kisline v različnih razvojnih fazah cveta. Vsebnost slednje je bila šestkrat večja v venčnih listov popkov v primerjavi s senescentnimi cvetovi, razlike pa so bile očitne tudi med delno odprtimi in popolnoma odprtimi cvetovi.

Tako kot spremembe vsebnosti antocianinov v venčnih listih razvijajočih cvetov vrtnic, razlike v vsebnosti fenolnih snovi lahko opišemo z več procesi. Biosinteza flavonolov je v nekaterih rastlinskih tkivih in organih razvojno regulirana, kot so opisali Noda in sod. (2004) pri *Eustoma grandiflora* (Raf.) Shinn. Vsebnost flavonolov se je v senescentnih cvetovih liziantusa namreč močno zmanjšala v primerjavi s popki, podobno kot je bilo ugotovljeno v raziskavi vrtnice 'KORcrisett' (Schmitzer in sod., 2009c). Hkrati so spremembe vsebnosti fenolov neposredno povezane z zmanjšanjem aktivnosti encimov PAL, CHI in DFR in drugih transkripcij genov kot poročajo Dong in sod. (1998) v petalih jablane. Podobna regulacija je verjetno prisotna tudi v venčnih listih vrtnic, kjer smo izmerili časovno spremembo vsebnosti fenolov glede na fazo cveta, zato bo v nadaljnjih študijah zanimivo analizirati vsebnosti nekaterih encimov in dopolniti znanja o modifikacijah, ki so razvojno pogojene.

Med barvnimi parametri, izmerjenimi s prenosnim kolorimetrom, in vsebnostjo prevladujočih ter skupnih antocianinov so bile prisotne tesne korelacije. Večje vrednosti parametra *a** so bile linearno povezane z večjimi vsebnostmi pelargonidin-3,5-di-glukozida, cianidin-3,5-di-glukozida in skupnih antocianinov. Podobna povezava je bila ugotovljena tudi med parametrom *b** in analiziranimi antocianini v venčnih listih vrtnice 'KORcrisett'. V obratno-sorazmerno zvezo pa so povezani barvni parameter *L** in prevladujoči ter skupni antociannii. Manj rdečih pigmentov je namreč vplivalo na učinek bledenja barve venčnih listov in s tem večje vrednosti *L**.

Avgusta 2009 smo v Arboretumu Volčji Potok vzorčili cvetove osmih sort pokrovnih vrtnic: tri bele sorte ('NOAschnee', 'KOReb', 'Sea Foam'), tri rdeče sorte ('POUleas', 'MORedfar', 'Gärtnerfreude') in dve rožnati sorti ('KORverlandus', 'The Fairy'). Spremljali smo spremembe obarvanosti venčnih listov in vsebnosti izbranih sekundarnih metabolitov v štirih fazah razvoja cvetov, da bi analizirali proces staranja cveta pri različno obarvanih sortah. Rezultate, ki smo jih ovrednotili pri razvoju cvetov miniaturne vrtnice 'KORcrisett' smo dopolnili z meritvami spremembe pH vrednosti celičnega soka, deleža vode v venčnih listih in njihovo svežo ter suho maso.

Sveža masa venčnih listov je bila praviloma statistično značilna najmanjša v fazi popka, se pri delno in popolnoma odprtih cvetovih povečala in zopet upadla v senescentnih cvetovih (Schmitzer in sod., 2010). Tudi Sood in sod. (2006) podajajo podobne rezultate v študiji razvoja cvetov pri vrtnicah *Rosa damascena* in *Rosa bourboniana*, vendar pa so proces staranja spremljali le do faze popolnoma odprtega cveta.

Povečanje sveže mase je bilo linearno povezano s povečanjem deleža vode v venčnih listih, ki je bil statistično značilno najmanjši v fazi popka in se povečal pri delno in popolnoma odprtih cvetovih. Največje razlike smo izmerili pri sorti 'NOAschnee', kjer je bil delež vode v popkih le 23 %, v senescentnih cvetovih pa smo izmerili 85 % delež vode. V senescentnih cvetovih smo praviloma izmerili zmanjšanje deleža vode pri vseh sortah razen pri že omenjeni beli sorti 'NOAschnee' in rdeči sorti 'MORedfar', kjer je bil delež

vode prav tako večji v primerjavi s popolnoma odprtimi cvetovi. Rezultati nakazujejo, da je povečanje celic in posledično velikosti delno in popolnoma odprtega cveta v primerjavi s popkom, v veliki meri odvisno od kopičenja vode v vakuolah. Relativno velik delež vode v senescentnih cvetovih in manjšo vrednost sveže mase venčnih listov lahko delno razložimo s strukturnim razpadom celične stene, kot sta pri nageljnih opisala De Vetten in Huber (1990). Posledično je bila izmerjena suha masa venčnih listov najmanjša pri senescentnih cvetovih kar deloma lahko razložimo z zmanjšano sintezo in akumulacijo metabolitov (Schmitzer in sod., 2010).

Spremembe pH vrednosti celičnega soka so bile z razvojem cveta pri vseh analiziranih sortah pokrovnih vrtnic statistično značilne – najmanjše vrednosti (od 4,6 do 5,3) so bile izmerjene v venčnih listih popkov, največje (nad 5,5) v senescentnih cvetovih. Podobne spremembe pH vrednosti so v starajočih venčnih listih vrtnic 'Mercedes' in 'Sweet Promise' izmerili tudi Barthe in Vaillant (1993) in Oren-Shamir in sod. (2001). Tudi Kuiper in sod. (1996) so izmerili vsebnost prostega amonija v celičnem soku in ga neposredno povezali s povišanjem pH vrednosti v senescentnih petalih vrtnic. Rahlo kisel vakuolni pH je značilen za rastlinske celice in že majhne spremembe vplivajo na stabilnost pigmentov in drugih sekundarnih metabolitov ter posledično na barvo tkiv okrasnih rastlin (Tanaka in sod., 2005).

Barvni parameter *a** je bil pri belih sortah pokrovnih vrtnic v vseh razvojnih stopnjah negativen, razlike med posameznimi fazami so bile majhne in niso bile skladne pri vseh analiziranih sortah. Tako so bile najmanjše vrednosti parametra *a** izmerjene v fazi popka pri sortah 'NOAschnee' in 'Sea Foam' in v fazi popolnoma odprtega cveta pri sorti 'KOReb'. Glede na izrazito majhno vsebnost antocianinov v cvetovih belih sort, ki je neposredno povezana z barvnim parametrom *a**, so dobljeni rezultati pričakovani. Po drugi strani, so bile pri rožnatih in rdečih sortah vrtnic največje vrednosti parametra *a** izmerjene v venčnih listih popkov in delno odprtih cvetov, najmanjše pa v senescentnih cvetovih. Podobne rezultate sta v študiji rezanega cvetja objavila tudi Marousky in Carlyle (1985), ki sta opisala manjše vrednosti parametra *a** pri senescentnih cvetovih vrtnice 'Better Times'. Vrednosti parametra *a** so bile v primerjavi z vrednostmi belih sort nekoliko večje pri svetlo rožnati sorti 'The Fairy' in še večje pri intenzivneje obarvanih sortah 'POUleas' in 'MORedfar'. Največje vrednosti parametra *a** so bile izmerjene na površini popkov sorte 'Gärtnerfreude', ki je tudi vizualno najbolj rdeče obarvana med analiziranimi sortami.

Pri vseh analiziranih sortah pokrovnih vrtnic smo izmerili podobno zmanjšanje vrednosti parametra *b** od faze popka do senescentnega cveta. Z razvojem in staranjem venčnih listov je bil pri rožnatih in rdečih sortah vrtnic opazen prehod v modrikaste in vijoličaste barvne odtenke, pri belih sortah pa kljub manjšim vrednostim parametra *b** prehoda barve nismo zaznali. Tudi Itzhaki in sod. (1990) so pri vrtnici 'Mercedes' vizualno opisali prehod intenzivne rdeče barve v modrikasto v zadnji razvojni fazi cveta, vendar pa sprememb kolorimetrično niso ovrednotili. Modrikast odtenek senescentnih cvetov rožnatih in rdečih sort vrtnic je dokazljivo povezan z večjo pH vrednostjo celičnega soka, ki vpliva na strukturne spremembe antocianinov (Cooper-Driver, 2001; Oren-Shamir in sod., 2001). Asen in sod. (1971) namreč poročajo o tvorbi kvercetinskih kompleksov s cianidin-3,5-di-glukozidom in posledičen prehod v modro barvo pri povečani pH vrednosti vakuolnega soka pri staranju vrtnice 'Better Times'. Pri vseh analiziranih sortah smo izmerili tesne

korelacije med barvnim parametrom *b** in pH vrednostjo celičnega soka; korelacije so bile potrjene tudi pri belih sortah.

Vrednost parametra *L** (svetlost) je bila pri vseh sortah najmanjša v popkih, ki so pri rožnatih in rdečih sortah tudi vizualno najbolj temni. Najmanjše vrednosti so bile izmerjene na površini venčnih listov popkov sorte 'Gärtnerfreude', kjer smo izmerili tudi največje vrednosti parametra *a**. Med izmerjenima barvnima parametroma *a** in *L** pri vrtnicah lahko opredelimo obratno sorazmerno povezavo, saj več rdečih pigmentov v vakuolah vpliva na večjo vrednost parametra *a** in ta na manjšo vrednost parametra *L**.

Slika 3: Prehod barve od rožnato obarvanih popkov (levo), roza delno in popolnoma odprtih cvetov in bledenja senescentnih cvetov vrtnice 'The Fairy'
Figure 3: Color changes of 'The Fairy' rose from intense rose buds (left), rose partially and fully open flowers to paler senescent flowers

Pri vseh analiziranih sortah vrtnic smo s pomočjo visokoločljivostne tekočinske kromatografije potrdili prisotnost dveh prevladujočih antocianinov: pelargonidin-3,5-di-glukozida in cianidin-3,5-di-glukozida kot v prejšnji študiji vrtnice 'KORcrisett' (Schmitzer in sod., 2009c). Pelargonidin-3-glukozid, cianidin-3-glukozid in peonidin-3-glukozid so bili prisotni le v petalih rdečih in rožnatih sortah pokrovnih vrtnic in njihova vsebnost ni pomembno vplivala na vsebnost skupnih antocianinov. Rdeči pigmenti so bili kvantificirani tudi v venčnih listih belih sort vrtnic, vendar so bile vsebnosti zelo majhne (Schmitzer in sod., 2010).

Z razvojem cveta je vsebnost prevladujočih in skupnih antocianinov naraščala od popka do popolnoma odprtega cveta in se rahlo zmanjšala v senescentnih cvetovih. Podobno zmanjšanje je bilo dokazano pri vseh analiziranih sortah, vendar pa spremembe niso bile vedno statistično značilne. Pri nekaterih sortah je bilo zmanjšanje vsebnosti skupnih antocianinov potrjeno že v popolnoma odprtih cvetovih, podobno kot poročajo Sood in sod. (2006), ki so spektrofotometrično analizirali vsebnost antocianinov v venčnih listih

razvijajočih cvetov vrtnice *R. damascena* in *R. bourboniana*. Ugotovili so namreč, da prvim začetnim fazam sinteze in kopičenja antocianinov v popkih in delno odprtih cvetovih sledi zmanjšanje vsebnosti pigmentov že v delno in popolnoma odprtih cvetovih, senescentnih venčnih listov pa niso analizirali. Manjša vsebnost antocianov v zadnjih fazah razvoja cveta je povezana s povišanjem pH vrednosti celičnega soka, ki bodisi vpliva na manjšo stabilnost antocianov in pospešeno delovanje razgradnih encimov kot so peroksidaze in β-glukozidaze ali na spremenjeno strukturo pigmentov v bolj bazičnem okolju (Oren-Shamir in sod., 2001). Podobni procesi so verjetno prisotni tudi v venčnih listih analiziranih sort vrtnic, saj je bila pH vrednost pri vseh sortah večja v popolnoma odprtih in senescentnih cvetovih. Med vsebnostjo skupnih antocianinov in barvnim parametrom *a** so bile pri vseh sortah potrjene linearne povezave, ki so bile statistično najbolj značilne in najtesnejše pri rožnatih in rdeče obarvanih sortah pokrovnih vrtnic.

V venčnih listih pokrovnih sort vrtnic smo analizirali vsebnosti šestih flavonolov iz skupine kvercetinov: kvercetin-3-rutinozid, kvercetin-3-galaktozid, kvercetin-3-glukozid, kvercetin-3-arabinozid, kvercetin-3-ksilozid in kvercetin-3-ramnozid. Prevladujoča kvercetin-3-ramnozid in kvercetin-3-glukozid sta predstavljala kar 90 % skupnih kvercetinov, kar so v raziskavi na številnih vrstah vrtnic potrdili tudi Mikanagi in sod. (1995). Najmanjše vsebnosti skupnih kvercetinov smo določili v popkih večine analiziranih sort, v nadaljnjih fazah razvoja cveta pa so se kvercetini kopičili v venčnih listih in njihova vsebnost je bila pri sorti 'NOAschnee' celo največja v senescentnih cvetovih.

Vsebnost skupnih fenolov pa je bila po drugi strani v senescentnih cvetovih analiziranih sort najmanjša (Schmitzer in sod., 2010), kar bi lahko povezali z zmanjšanjem vsebnosti skupnih antocianinov in tudi drugih fenolnih spojin, ki jih v raziskavi nismo zajeli. Manjše vrednosti skupnih fenolov v zadnjih fazah razvoja cveta pri vrtnici domnevno vplivajo na slabšo antioksidativno obrambo celic, ki postanejo bolj občutljive na oksidativni stres (Takahama in Oniki, 1997). Senescentni venčni listi vrtnic so podvrženi procesom oksidacije in posledično programirani celični smrti (Sood in sod., 2006) z razpadom membrane in lipidov. V raziskavi smo pri osmih sortah pokrovnih vrtnic dokazali zmanjšanje vsebnosti skupnih fenolov ter zmanjšanje sveže in suhe mase v senescentnih cvetovih in tako osvetlili proces staranja cvetov vrtnic. Selektivna in pravočasna izbira začetnih intenzivneje obarvanih razvojnih faz cvetov pri vrtnicah je v pigmentni industriji zato nadvse pomembna.

V naslednjem poskusu smo preučevali vpliv pH vrednosti substrata na količino antocianinov, kvercetinov, katehina in fenolnih kislin v venčnih listih popolnoma odprtih cvetov vrtnice *Rosa* x *hybrida* 'KORcrisett'. Miniaturne vrtnice so običajno sajene v rahlo kisle substratne mešanice (pH vrednost okoli 6), zanimalo pa nas je, kako spremenjene pH vrednosti substrata vplivajo na vsebnosti sekundarnih metabolitov, saj podatkov o tvorbi antocianov in barvi cvetov v različnih kislostih substrata v literaturi nismo zasledili. Določili smo tri obravnavanja: pH vrednost 3,3 (kisel substrat), pH vrednost 4,7 (kontrola) in pH vrednost 7,3 (bazičen substrat). Obenem smo spremljali vpliv pH vrednosti substratne mešanice na tvorbo cvetov.

Pri začetnem vzorčenju smo na posamezni rastlini miniaturne vrtnice prešteli 9,3 cvetove (upoštevali smo število popkov, delno odprtih in popolnoma odprtih cvetov ter senescentnih cvetov), po 28 dneh pa smo v različnih substratnih mešanicah izmerili značilne spremembe števila cvetov. Rastline, ki so bile posajene v substratni mešanici s pH vrednostma 3,3 in 7,3, so po 28 dneh tvorile statistično značilno manj cvetov od kontrolnih rastlin, kjer smo v povprečju prešteli 13,5 cvetov na posamezni rastlini (Schmitzer in Štampar, 2010). Zmanjšanje števila cvetov pri vrtnicah, posajenih v izrazito kisel (v povprečju 8,5 cvetov na rastlini) in rahlo bazičen substrat (v povprečju 9,2 cvetov na rastlini), bi lahko povezali s spremenjeno dostopnostjo hranil, kot poročajo Smith in sod. (2004), ki so preučevali vpliv pH vrednosti substrata na privzem hranil pri *Impatiens* in *Petunia* hibridih. Podobno je bila tvorba cvetov pri *Senecio vulgaris* manjša pri rastlinah, kjer je bil privzem hranil iz substrata manjši (Brown in Molyneux, 1996), pri tobaku pa so zaznali celo spremembe v terminu cvetenja povezane z izrazito kislimi ali bazičnimi pH vrednostmi substrata (Pasqua in sod., 1991).

Prav tako smo v venčnih listih vrtnic 'KORcrisett', ki so bile posajene v različne pH vrednosti substrata, izmerili spremembe v vsebnosti nekaterih sekundarnih metabolitov. Vsebnost prevladujočih antocianinov pelargonidin-3,5-di-glukozida in cianidin-3,5-di-glukozida je bila po 28 dneh najmanjša v venčnih listih kontrolnih rastlin. Pri rastlinah sajenih v izrazito kisel in rahlo bazičen substrat je bila vsebnost pelargonidin-3,5-di-glukozida 64 % in 67 % večja od kontrole in vsebnost cianidin-3,5-di-glukozida 70 % in 60 % večja (Schmitzer in Štampar, 2010). Največje statistično značilne razlike med obravnavanji pH vrednosti substrata pa so bile izmerjene v vsebnosti pelargonidin-3-glukozida, ki je bila kar dvakrat večja v venčnih listih vrtnic, sajenih v pH 3,3 in 7,3 v primerjavi s kontrolo.

Posledično je bila pri drugem vzorčenju izmerjena 61 % večja vsebnost skupnih antocianov v petalih rastlin, posajenih v substratno mešanico s pH vrednostjo 7,3 in 67 % večja vsebnost v izrazito kislem substatu v primerjavi s prvim vzorčenjem in kontrolo, predvsem na račun povečanja vsebnosti cianidin-3,5-di-glukozida (Schmitzer in Štampar, 2010). Vsebnosti pelargonidin-3,5-di-glukozida in treh manj zastopanih antocianinov (pelargonidin-3-glukozida, cianidin-3-glukozida in peonidin-3-glukozida) so bile pri prvem in drugem vzorčenju podobne; pH vrednost substratne mešanice namreč ni značilno vplivala na njihovo vsebnost v venčnih listih vrtnice 'KORcrisett'. Spremembe v kopičenju antocianinov v izrazito kislem in rahlo bazičnem substratu so verjetno povezane s stresom, kot je opisala Hawrylak-Nowak (2008) pri koruzi. Podobno so bile večje vsebnosti karotenoidov v venčnih listih *Impatiens* in *Petunia* hibridov analizirane v substratih kislih pH vrednosti (Smith in sod., 2004).

Podobno kot na vsebnost antocianinov je pH vrednost substratne mešanice vplivala na vsebnosti kvercetinov, katehina in fenolnih kislin. Najmanjše vsebnosti kvercetin-3-ramnozida, kvercetin-3-rutinozida in kvercetin-3-glukozida so bile pri drugem vzorčenju analizirane v venčnih listih vrtnic, posajenih v substrat s pH vrednostjo 4,7, največje pa v substrat s pH vrednostjo 7,3. Pri slednjem so bile vsebnosti prevladujočih kvercetin-3-ramnozid in kvercetin-3-glukozida v venčnih listih dvakrat večje kot pri kontrolnem obravnavanju in v primerjavi s prvim terminom vzorčenja (Schmitzer in Štampar, 2010). Vsebnost katehina ni bila v tolikšni meri odvisna od pH vrednosti substratne mešanice, saj

so bile večje vsebnosti pri drugem vzorčenju izmerjene le v obravnavanju substrata pH vrednosti 3,3, med substratom pH vrednosti 4,7 in 7,3 pa ni bilo zaznati statistično značilnih razlik. Po drugi strani je pH vrednost substrata značilno vplivala na vsebnosti galne, protokatehulne, kavne in *p*-kumarne kisline. Vsebnosti so bile pri drugem vzorčenju največje v izrazito kislem substratu pH 3,3.

3.2 SKLEPI

V raziskavi povezanosti barvnih parametrov in vsebnosti fenolnih snovi smo izvedli številne poskuse in vzorčenja, da bi odgovorili na zastavljene hipoteze. Študija, v kateri smo predstavili korelacijo med prevladujočim antocianinom v listih pahljačastih javorjev in CIE LAB parametri na treh položajih na drevesu, podaja tesno zvezo med postopno jesensko obarvanostjo listov in topofizo. Določitev barvnih parametrov v štirih razvojnih fazah različnih sort vrtnic in njihova povezava z vsebnostjo izbranih sekundarnih metabolitov dopolni podatke o vizualni spremembi barve s staranjem cvetov pri rdečih in rožnatih sortah. V sklopu poskusov smo prišli do naslednjih ugotovitev:

- Jesenska rdeča obarvanost listov pahljačastih javorjev je odvisna od sorte in položaja lista na drevesu. Vrednost barvnega parametra *a**, ki določa intenzivnost rdeče obarvanosti lista, je bila največja v listih z vrha krošnje in najmanjša v listih z vej, ki so izraščale s spodnjega dela provodnika.

- Prevladujoča antocianina v listih pahljačastih javorjev sta cianidin-3-glukozid in cianidin-3-rutinozid. Njuna vsebnost je odvisna od sorte in pozicijsko pogojena. Največje vsebnosti prevladujočega cianidin-3-glukozida smo izmerili v listih z vrha krošnje, najmanjše v listih z vej, ki so izraščale s spodnjega dela provodnika. Cianidin-3-glukozid v jesenskih listih pahljačastih javorjev predstavlja količinski biokemijski parameter določen s položajem lista na drevesu (topofizo).

- Cianidin-3-glukozid, je v tesni korelaciji s CIE LAB barvnimi parametri *a**, *b**, *h*° in *L**. Izražanje rdeče barve pri pahljačastih javorjih je močno povezano z vsebnostjo prevladujočega antocianina v listih, manj pa z vsebnostjo drugih izmerjenih rdečih pigmentov.

- Barva cvetov vrtnice 'KORcrisett' je pogojena s starostjo venčnih listov. Vrednost barvnega parametra *L** (svetlost) se z razvojem in staranjem cveta poveča. Statistično značilno je na isti rastlini najtemnejši popek, delno odprt in popolnoma odprt cvet sta svetlejša, senescentni cvetovi so najsvetlejši.

- V venčnih listih vrtnice 'KORcrisett' prevladujeta antocianina pelargonidin-3,5-di-glukozid in cianidin-3,5-di-glukozid. Njuna vsebnost je največja v popkih in najmanjša v senescentnih cvetovih. Vsebnost manj zastopanih pelargonidin-3-glukozida, cianidin-3-glukozida in peonidin-3-glukozida je prav tako največja v popkih in najmanjša v senescentnih cvetovih. Večje vrednosti parametrov *a** in *b**

so linearno povezane z večjimi vsebnostmi pelargonidin-3,5-di-glukozida, cianidin-3,5-di-glukozida in skupnih antocianinov. V obratno-sorazmerni zvezi so povezani barvni parameter *L** in prevladujoči ter skupni antocianini.

- V venčnih listih vrtnice 'KORcrisett' se z razvojem cveta vsebnost prevladujočih kvercetin-3-ramnozida in kvercetin-3-rutinozida močno zmanjša. Podoben trend je zaznati tudi v vsebnosti katehina ter kavne, klorogenske, galne, protokatehulne in *p*-kumarne kisline.

- Sveža masa venčnih listov analiziranih pokrovnih sort vrtnic je statistično značilno najmanjša v fazi popka, pri delno in popolnoma odprtih cvetovih je večja in se zopet zmanjša v senescentnih cvetovih. Povečanje sveže mase je linearno povezano s povečanjem deleža vode v venčnih listih. Suha masa venčnih listov je najmanjša v fazi senescentnih cvetov. Najnižje pH vrednosti (od 4,6 do 5,3) so bile izmerjene v venčnih listih popkov, najvišje (nad 5,5) v senescentnih cvetovih.

- Pri rožnatih in rdečih sortah vrtnic so bile največje vrednosti parametra *a** izmerjene v venčnih listih popkov in delno odprtih cvetov, najmanjše v senescentnih cvetovih. Pri belih sortah vrtnic podobnega zmanjšanja ni zaznati. Vrednost parametra *L** (svetlost) je bila pri vseh sortah najmanjša v popkih in največja v senescentnih venčnih listih. Vrednost parametra *b** se od faze popka do senescentnega cveta zmanjša. Tesne korelacije med barvnim parametrom *b** in pH vrednostjo celičnega soka so bile potrjene pri vseh analiziranih sortah pokrovnih vrtnic.

- Prevladujoča antocianina pelargonidin-3,5-di-glukozid in cianidin-3,5-di-glukozid sta bila prisotna v venčnih listih vseh analiziranih sort pokrovnih vrtnic (tudi belih); z razvojem cveta je vsebnost prevladujočih in skupnih antocianinov naraščala od popka do popolnoma odprtega cveta in se zmanjšala v senescentnih cvetovih. Med barvnim parametrom *a** in skupnimi antociannii so bile izmerjene tesne korelacije.

- Kvercetin-3-rutinozid, kvercetin-3-galaktozid, kvercetin-3-glukozid, kvercetin-3-arabinozid, kvercetin-3-ksilozid in kvercetin-3-ramnozid se z razvojem cveta kopičijo v venčnih listih. Vsebnost skupnih fenolov je bila najmanjša v senescentnih cvetovih analiziranih sort.

- Pri vrtnicah 'KORcrisett', posajenih v izrazito kislo (3,3) in rahlo bazično (7,3) substratno mešanico, smo opazili značilno zmanjšano tvorbo cvetov v primerjavi z rastlinami, posajenimi v substratno mešanico rahlo kisle pH vrednosti(4,7).

- Vsebnost prevladujočih in skupnih antocianinov v venčnih listih vrtnice 'KORcrisett' je bila najnižja pri rastlinah, posajenih v substrat s pH vrednostjo 4,7 in izrazito višja pri izrazito kisli in rahlo bazični pH vrednosti substrata. pH

vrednost substratne mešanice je vplivala na vsebnosti kvercetinov, katehina in fenolnih kislin, ki so bile najmanjše pri pH vrednosti substrata 4,7 in večje pri pH vrednosti 3,3 in 7,3.

Proces postopnega jesenskega obarvanja listov je dokaj neraziskan in veljalo bi razširiti raziskavo o učinku topofize na primerjavo vsebnosti drugih sekundarnih metabolitov in encimov v listih ter jih povezati z različnimi morfološkimi in fiziološkimi spremembami (npr. sposobnostjo koreninjenja pahljačastih javorjev). S tem bi dopolnili spekter biokemijskih parametrov, ki ovrednotijo pozicijski učinek pri lesnatih rastlinah. V prihodnjih študijah na pahljačastih javorjih bi se bilo prav tako zanimivo osredotočiti na specifične morfološke spremembe, kot so velikost celic, na vsebnost vode in pH celičnega soka ter določiti vsebnosti nekaterih fenolnih snovi, ki sodelujejo pri barvi. Tako bi še natančneje ovrednotili spremembe, ki se zgodijo v jesenskem času, in vplivajo na postopen razvoj rdeče in rumene obarvanosti listov lesnatih rastlin.

Ugotovitve o spremembi barve in vsebnosti fenolnih snovi s staranjem cveta vrtnice nakazujejo, da sta sinteza in razgradnja rdečih pigmentov razvojno pogojeni. V nadaljnje analize bi bilo smotrno vključiti spremljanje aktivnosti encimov, ki igrajo ključno vlogo v sintezi antocianinov in ugotoviti, kako se njihova aktivnost spreminja glede na posamezno razvojno fazo. Obenem bi veljalo nadaljevati raziskave vpliva pH vrednosti substratne mešanice na morfološke in biokemijske spremembe miniaturnih vrtnic in s tem določiti optimalne pogoje za njihovo cvetenje in hkrati visoko vsebnost pigmentov in drugih fenolnih snovi v njihovih petalih.

4 POVZETEK (SUMMARY)

4.1 POVZETEK

V poskusih na pahljačastih javorjih in vrtnicah smo spremljali parametre, ki vplivajo na izražanje barve pri različnih organih okrasnih rastlinah. Pri sedmih sortah *Acer palmatum* Thunb. smo določili tri obravnavanja, glede na položaj veje na drevesu, in vzorčili jesensko obarvane liste. Merili smo barvne parametre, analizirali sestavo prevladujočih antocianinov in rezultate povezali s korelacijsko analizo.

Pri sorti miniaturne vrtnice in osmih sortah pokrovnih vrtnic smo spremljali razvoj cveta in v štirih fazah merili barvne parametre, fiziološke spremembe, v venčnih listih kvantificirali vsebnosti prevladujočih sekundarnih metabolitov in določene parametre povezali s korelacijsko analizo. Pri miniaturni sorti vrtnice smo obenem spremljali vpliv pH vrednosti substratne mešanice na tvorbo cvetov in vsebnost izbranih sekundarnih metabolitov v venčnih listih.

Ugotovili smo, da je postopna jesenska rdeča obarvanost listov pahljačastih javorjev sortno in pozicijsko pogojena. Intenzivnost rdeče obarvanosti je bila pri vseh analiziranih sortah pahljačastih javorjev največja v listih z vrha krošnje in najnižja v listih z vej, ki so izraščale s spodnjega dela provodnika. Prav tako je bila vsebnost prevladujočega antocianina cianidin-3-glukozida največja v listih z vrha krošnje, najmanjša pa v listih z vej, ki so izraščale s spodnjega dela provodnika. Zato cianidin-3-glukozid v jesenskih listih pahljačastih javorjev predstavlja količinski biokemijski parameter določen s položajem lista na drevesu (topofizo). Korelacijske analize so potrdile povezanost barvnih parametrov z vsebnostjo prevladujočega antocianina v listih vseh analiziranih sort pahljačastih javorjev.

Na barvo cvetov analiziranih sort vrtnic poleg vsebnosti pigmentov močno vpliva tudi starost venčnih listov. Svetlost cvetov se z razvojem in staranjem poveča, prav tako je pri rdeče in rožnato obarvanih sortah zaznati zmanjšanje rdeče obarvanosti in prehod v modrikaste tone v zadnji fazi senescentnega cveta. Statistično značilno je na isti rastlini vedno najtemnejši popek, delno odprt in popolnoma odprt cvet sta praviloma svetlejša, senescentni cvetovi pa najsvetlejši. S staranjem cveta se do faze popolnoma odprtih cvetov povečata sveža in suha masa venčnih listov ter delež vode in se nato v senescentnih cvetovih zmanjšajo. Spremembe pH vrednosti celičnega soka so izrazite v senescentnih cvetovih, kjer smo zaznali povečanje pH vrednosti za eno enoto pri vseh analiziranih sortah. Prehod v modre odtenke senescentnih cvetov je povezan z večjo pH vrednostjo celičnega soka.

V venčnih listih analiziranih sort vrtnic prevladujeta antocianina pelargonidin-3,5-di-glukozid in cianidin-3,5-di-glukozid, potrdili smo prisotnost manj zastopanih pelargonidin-3-glukozida, cianidin-3-glukozida in peonidin-3-glukozida. Vsebnost prevladujočih in

skupnih antocianinov narašča od popka do popolnoma odprtega cveta in se zmanjša v senescentnih cvetovih. Med barvnim parametrom *a** (količina rdeča obarvanosti) in skupnimi antocianini so bile pri vseh sortah prisotne tesne korelacije.

Najmanjše vsebnosti skupnih kvercetinov smo določili v popkih večine analiziranih sort, v nadaljnjih fazah razvoja cveta so se kvercetin-3-rutinozid, kvercetin-3-galaktozid, kvercetin-3-glukozid, kvercetin-3-arabinozid, kvercetin-3-ksilozid in kvercetin-3-ramnozid kopičili v venčnih listih. Vsebnost katehina, kavne, klorogenske, galne, protokatehulne in *p*-kumarne kisline ter skupnih fenolov pa je bila v senescentnih cvetovih analiziranih sort vrtnic najmanjša.

Pri miniaturni vrtnici, ki smo jo posadili v izrazito kislo (3,3) in rahlo bazično (7,3) substratno mešanico, smo opazili značilno zmanjšano tvorbo cvetov v primerjavi z rastlinami, sajenimi v substratno mešanico rahlo kisle pH vrednosti (4,7). Po drugi strani pa je bila vsebnost prevladujočih in skupnih antocianinov v venčnih listih najmanjša pri rastlinah, posajenih v substrat pH vrednosti 4,7 in izrazito višja pri rastlinah, posajenih v substrat pH vrednosti 3,3 in 7,3. Podobno povečanje vsebnosti kvercetinov, katehina in fenolnih kislin smo izmerili pri rastlinah, posajenih v substrat pH vrednosti 3,3 in 7,3.

4.2 SUMMARY

Parameters affecting the expression of color in various organs of ornamental plants were monitored on Japanese maple and roses. According to the branch position on the tree, three treatments were established in seven *Acer palmatum* Thunb. cultivars and autumn leaves were sampled. Color parameters were monitored, the composition of the predominant anthocyanins analyzed and the results linked with correlation analysis.

Flower development was monitored in a cultivar of miniature rose and eight cultivars of groundcover roses. Four developmental stages were determined and color parameters, physiological changes, as well as the predominant secondary metabolites were analyzed and linked with correlation analysis. Additionally, the effect of substrate pH level on the formation of flowers and the content of selected secondary metabolites in petals was monitored in a miniature rose cultivar.

It was determined that the gradual autumn coloration of Japanese maple leaves is cultivar and position dependent. The intensity of red coloration was always highest in leaves collected from branches from the top of the canopy and lowest in leaves of the base branches. Similarly, the content of the predominant anthocyanin cianidin-3-glucoside was highest in the leaves from the top of the canopy and lowest in the leaves from base branches. Therefore, cianidin-3-glucoside can be considered a quantitative biochemical marker of the positional effect in the autumn leaves of Japanese maple cultivars. Correlation analysis confirmed the relationship of color parameters with the predominant anthocyanin content in leaves of all cultivars analyzed.

In addition to pigment content, flower color of different rose cultivars is strongly dependent on the developmental stage of the flower. Flower lightness increases with the developmental stage and senescence. Also, in red and pink cultivars, a decrease of red coloration was confirmed and a transition to bluish hues in the final stage of the senescent flower noted. On individual plants, buds are always darkest, the color of partially open and fully open flowers is less intense and senescent flowers are the palest. With the progression of flower aging, fresh and dry weight of the petals as well as the percentage of water increases to the fully open flower stage. In senescent flowers a decrease of these parameters was measured. Changes in cellular sap pH level were most significant in senescent flowers, where an increase of pH level by one unit was detected in all rose cultivars analyzed. Transition to blue hues of the senescent flower was linked with a higher pH level of the cellular sap.

In the petals of analyzed roses cultivars two predominant anthocyanins were determined: pelargonidin-3,5-di-glucoside and cyanidin-3,5-di-glucoside. Additionally, the content of less abundant pelargonidin-3-glucoside, cyanidin-3-glucoside and peonidin-3-glucoside was analyzed. The content of the predominant and total anthocyanins increased from the bud to the fully open flower stage and then decreased in the senescent flowers. Tight correlations were established between the color parameter *a** (the level of red coloration) and total anthocyanins in all cultivars analyzed.

The lowest content of total quercetins was determined in the buds of most cultivars analyzed. Quercetin-3-rutinoside, quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-arabinoside, quercetin-3-xyloside and quercetin-3-ramnoside accumulated in the petals of the subsequent developmental stages. However, the content of catechin, caffeic, chlorogenic, gallic, protocatechulic and *p*-coumaric acid as well as total phenols was lowest in the petals of senescent flowers of all cultivars analyzed.

Po drugi strani pa je bil a vsebnost prevladujočih in skupnih antocianov v venčnih listih najmanjša pri pH substrata 4,7 in izrazito višja pri pH 3.3 in 7,3. Podoben trend povečanja vsebnosti kvercetinov, katehina in fenolnih kislin smo izmerili pri rastlinah, sajenih v pH substratne mešanice 3.3 in 7,3.

A decrease in the formation of flowers was detected in miniature rose plants, potted in highly acidic (3.3) and slightly alkaline (7,3) substrate mix, compared to plants, potted in a weakly acidic (4.7) substrate. On the other hand, the content of predominant and total anthocyanins was lowest in petals of plants, potted in substrate with pH level 4.7 and increased in petals of flowers, potted in 3.3 and 7.3 pH level. A similar trend of an increased content of quercetins, catechin and phenolic acids was observed in petals of flowers, potted in 3.3 and 7.3 pH level.

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ZAHVALA

Hvala trem najpomembnejšim m-jem: **mamici**, **možu** in **mentorju**.

Seveda tudi vsem drugim, ki ste s svojimi pripombami, potrpežljivostjo in spodbudo sodelovali pri zaključku mojega študijskega obdobja.

Naj vam bo misel Alberta Camusa pri srcu, ko se ozrete v tišino in moč narave!

Jesen je druga pomlad, ko je vsak list cvet.