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Flavonoids in flowers of 16 *Kalanchoë blossfeldiana* varieties

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Abstract

Kalanchoë blossfeldiana varieties with orange, pink, red and magenta flowers were found to contain 3,5-*O*-β-D-diglucosides of pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin. Pink, red and magenta varieties contained relatively high amounts of quercetin based flavonols. Four distinct quercetin flavonols were identified, namely quercetin 3-*O*-β-D-glucoside and three that were quercetin 3-*O*-α-L-rhamnoside based, with either glucose, xylose or arabinose attached to position 2 of the rhamnose. In addition, the presence of at least three kaempferol based diglycosides was suggested from LC–MS analyses. Orange varieties contained very low amounts of flavonol co-pigments and of delphinidin derivatives. The flower extracts of the varieties 'Diva' (magenta) and 'Molly' (red) had identical anthocyanin ratios but differed significantly in flavonol content. The magenta variety contained four times as much quercetin relative to anthocyanidin as the red variety. This difference was mainly due to a larger content of quercetin 3-*O*-(2″-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside). Based on pigment and co-pigment analyses, approaches for molecular breeding towards blue flower colour are discussed.

Keywords: *Kalanchoë blossfeldiana*; Crassulaceae; Flavonoids; Pelargonidin; Cyanidin; Peonidin; Delphinidin; Petunidin; Malvidin; Quercetin; Kaempferol; Rhamnose; Glucose; Arabinose; Xylose

1. Introduction

Flavonoid biosynthesis has been the subject of many studies and especially the anthocyanin pathway is well documented (Cooper-Driver, 2001; Winkel-Shirley, 2001). A general model of the anthocyanidin biosynthetic pathway was devised more than 10 years ago (Holton and Cornish, 1995). Subsequently, most work has focussed on the late biosynthetic steps involving mainly glycosylation and acylation of anthocyanins to form more complex anthocyanin molecules, e.g., to provide blue flower colour. These secondary modifications offer interesting prospects for molecular breeding (Mol et al., 1998).

No reports on the flavonoid content of *Kalanchoë blossfeldiana* have appeared since a pioneering study in 1963 (Neyland et al., 1963). This study identified cyanidin 3-*O*-glucoside; cyanidin 3,5-*O*-diglucoside and peonidin 3,5-*O*-diglucoside in flowers and leaves of the variety 'Tom Thumb'. Since then, breeding efforts have expanded the flower colour spectrum immensely, from the reddish orange varieties available in the sixties to the bright yellow, orange, pink, red, magenta and white varieties available today. The natural variation in flower pH remains unknown and the potential to obtain new flower colours by traditional breeding supported by modern LC analysis and molecular breeding have not been exploited.

This research communication reports identification of the anthocyanins and flavonol–glycosides present in 16 of the *K. blossfeldiana* varieties currently available and

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discusses plausible modifications of the general biosynthesis pathway to generate a blue flower colour.

2. Results and discussion

Sixteen *K. blossfeldiana* varieties were obtained from two commercial growers on the island of Fyn, Denmark. The varieties are listed in Table 1 together with their colour, flower pH, anthocyanidin and quercetin content.

The anthocyanidin, flavonol and flavone content of acid hydrolysed flower extracts were determined by LC and comparison with authentic standards. This revealed presence of very low amounts of pelargonidin, relatively high amounts of cyanidin and varying amounts of peonidin, delphinidin, petunidin and malvidin as illustrated for the two cultivars 'Diva' and 'Molly' (Fig. 1). Hydrolysed extracts often contained relatively high amounts of quercetin and small amounts of kaempferol. No myricetin and no flavones (apigenin, eriodictyol and tricetin) were detected.

To determine the structure of the anthocyanins, non-hydrolysed extracts were analysed using LC–MS. This established the parent ions $[M + H^+]$ of six anthocyanins as m/z 595, 611, 625, 627, 641 and 655. These masses correspond to the diglucosides of the anthocyanidins found. To establish the glycosylation pattern, retention times and UV-spectra of the components were compared with 3,5-*O*-β-D-diglucosides from *Crocus* 'Tricolor' (Norbaek and Kondo, 1998). The *Crocus* anthocyanins had identical chromatographical and UV-spectral characteristics to the

Table 1
Content of anthocyanidins and co-pigments in hydrolysed flower extracts from 16 varieties of *K. blossfeldiana*

Variety	Flower color	Flower pH $LSD_{0.95}$=0.11	Anthocyanidin µg/g fw	Quercetin µg/g fw	% relative to total anthocyanidin content $LSD_{0.95}$=8						
					% pel	% cyd	% peo	% del	% pet	% mal	% que
Titan	orange	4.40	57	1	5	88	0	7	0	0	1
Fame	red orange	4.60	206	2	5	83	9	3	0	0	1
Petero	red orange	4.65	289	3	1	96	3	1	0	0	1
Debbie	orange red	4.50	254	3	4	70	23	3	0	0	1
Altar	orange red	4.35	392	88	0	78	19	3	0	0	22
Sumaco	dark red	4.50	360	49	0	42	12	23	17	6	14
Molly	dark red	4.50	248	30	0	31	26	10	12	21	12
Diva [A]	magenta	4.50	125	65	0	39	22	15	9	14	52
Neon [a]	dark pink	4.55	93	60	0	56	36	8	0	0	65
Pinky [a]	pink	4.55	50	54	0	59	27	14	0	0	108
Cora [B]	pink	4.40	21	17	0	62	5	27	6	0	82
Dark Cora [b]	dark pink	4.30	55	23	0	59	9	21	11	0	41
Bromo [C]	magenta	4.65	155	99	0	22	12	24	17	24	64
Brava [c]	dark pink	4.50	99	82	0	47	30	15	3	5	83
Oriba	dark pink	4.55	158	10	1	76	13	7	3	0	6
Lican	magenta	4.65	124	26	0	21	16	21	17	25	21

del, delphinidin; cyd, cyanidin; pet, petunidin; pel, pelargonidin; peo, peonidin; mal, malvidin; que, quercetin. [a–c] indicates that the varieties are spontaneous mutants from the ancestors [A–C], respectively.