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Flavonoids in white and yellow perianths and yellow anthers of tulips (*Tulipa gesneriana* L.)

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Summary

The content of flavonoids in white and yellow perianths and yellow anthers of a few tulip cultivars were determined at the stage of full flowering. To analyses of flavonols a HPLC method was used. In anthers (yellow) of all analyzed cultivars (Oscar, Pax, Profesor Wóycicki, Biała Dama, White Virgin, Calypso, Diana) high content of quercetin (2.35 - 6.01 mg · g^{-1} F.W.), kaempferol (1.09 - 9.47 mg · g^{-1} F.W.) and apigenin (1.34 - 8.24 mg · g^{-1} F.W.) was found. In analyzed white perianth of cvs. Oscar and White Virgin also high content of quercetin (1.3 - 1.80 mg · g^{-1} F.W.) and kaempferol (1.90 mg · g^{-1} F.W.) was documented and only traces of apigenin was found. In the yellow perianth of cv. Profesor Wóycicki the level of quercetin and kaempferol was much lower than in perianth of cvs. Oscar and White Virgin, and apigenin was absent. Thus, yellow anthers and white and yellow perianth of tulip cultivars are a rich source of flavonols.

Key words: tulip, *Tulipa gesneriana,* flavonoids, perianth, anthers, quercetin, kaempferol, apigenin

INTRODUCTION

Flavonoids are a large group (more than 4000 of flavonoids have been reported) of naturally occurring compounds (Iwashina, 2000). Flavonols are flavonoids of particular importance as they are have been found to posses antioxidant and free radical scavenging activity (Pietta, 2000). Among them flavonols (flavan-3,4-diols) - quercetin, kaempferol and myricetin are widely distributed within the plant kingdom (Harborne, 1994; Formica and Regelson, 1995).

Flavonoids take part in defense system against attack of pathogens, insects and stress conditions during plant growth and development (Harborne, 1994; Tomas-Barberan et al., 1988). They play important role in auxin biosynthesis and are responsible for colour and taste of plant tissues as well (Moore, 1989). Therefore composition and concentration of flavonoids can attracts or repels the insects as a flower pollinators (Hedin and Wangea, 1986). It was found too that flavonoids protect plants against free radicals and oxidative damages caused by UV radiation present in sunlight (Larson, 1988).

Vegetables, fruits and beverages are the main dietary sources of flavonols, primarily as quercetin and kaempferol (Herrmann, 1988; Toyoda et al., 1997; Marken and Beecher, 2000). One of the major sources of flavonols in the European diet is the onion and some other *Allium* crops (Hertog et al., 1992; Horbowicz, 2000). Among onion cultivars high level of quercetin glycosides were found in brown-skinned and red-skinned cultivars. In fleshy scales of brown-skinned onions cultivated in Poland, the concentration of quercetin ranged from 0.298 to 0.828 mg $\cdot$ g^{-1} of fresh weight (Horbowicz and Kotlińska, 1998). Exceptionally rich in quercetin (mainly in free form) are dry brown and red outer scales of onion (10 - 30 mg $\cdot$ g^{-1} dry weight). White cultivars of onion (dry outer scales as well as fleshy scales) contain only traces of quercetin (Horbowicz, 2000).

Colours of onion dry scales (deep yellow or light brown) and inner fleshy scales (light yellow) is caused by quercetin in free form, and glycosides of quercetin, respectively. Pure aglycones and glycosides of quercetin, kaempferol and apigenin have yellow colour or light brown.

Colour of plant tissues is a composition of three main classes of natural pigments: carotenoids, chlorophylls and flavonoids. In blooming flowers occur carotenoids, anthocyanins and flavonols glycosides.

Different flavonols were identified in tulip (*Tulipa gesneriana* L.) organs, leaves, perianth and pollen (Strack et al., 1981; Budzianowski, 1991). However, most literature concerns identification and measurements of anthocyanidins in tulip perianths, anthers and pollens (Nakayama et al., 1999; Nieuwhof et al., 1990; Van Raamsdonk, 1993; Halevy and Asen, 1959; Shibata and Ishikura, 1959; Shibata and Yoshitama, 1968; Shibata and Sakai, 1961; Torskangerpoll et al., 1999).

The aim of this work was to determine the content of some flavonoids in perianths and anthers of a few white and yellow tulip cultivars.

MATERIAL AND METHODS

Plant material

The perianths (white and yellow) and anthers (yellow) from a few cultivars of tulip (*Tulipa gesneriana* L.) (Tab. 1) were collected separately at full flowering from the garden of the Research Institute of Pomology and Floriculture at Skierniewice, Poland.

Description of chemical analyses

The flavonols content was determined according to method based on P a t i l et al. (1995) procedure with many own modifications (H o r b o w i c z, 1999). Twenty plants were taken to analyses. After knife cutting a 20 g samples of perianths tissue were blended with 10-fold (v/w) portion of 60% ethanol-water solution (in case of anthers 0.5 g samples were homogenized with 20-fold volume of 60% ethanol-water). Followed the samples were kept overnight at ambient temperature, and then filtered over medium speed filter paper. To 1.5 ml aliquots placed in screw-capped vials 0.5 ml 6N HCl was added, and flavonoid glycosides were hydrolysed at 100°C during 30 min. Then one ml of water was added to hydrolysate and obtained aglycones were extracted by three times vigorous shaking with 1 ml of ethyl acetate. Separated upper layer was withdrawn using Pasteur pipette. Pooled acetate layers were diluted with methanol : water solution (1:1, v/v).

HPLC conditions

To analyses of flavonols a LKB (Sweden) HPLC apparatus equipped with Rheodyne 7125 injection system (20 µl loop), UV detector (2151 Variable Wavelength Monitor) set at 370 nm, and Shimadzu C-R6A Chromatopac integrator was used. The flavonols were isocratically separated on Lichrosorb RP18 (4 x 250 mm, 10 µm) column, and a mobile phase was methanol: water mixture (55:45, v/v) contained 0.2% ortho-phosphoric acid. The flow rate was 0.8 ml/min.

Standards of flavonoid aglycons for analysis were purchased from Sigma (quercetin) and Fluka (kaempferol and apigenin). The standard curves was prepared for concentration range 0.2 to 10.0 µg/ml.

Analyses were done in three replicates, and results were statistically calculated by use t-Student (standard deviation).

RESULTS AND DISCUSSION

White and yellow perianths of investigated tulip cultivars contained a relatively high level of quercetin and kaempferol glycosides, and traces of apigenin only (Fig. 1).

Table 1. Contents of flavonoids in yellow anthers of several tulip cultivars.

Cultivar	Colour of perianths	Quercetin	Kaempferol	Apigenin	Total
		contents ($mg \cdot g^{-1}$ of fresh weight ± SD)			
Oscar	white	4.27 ± 0.23	5.58 ± 0.22	2.53 ± 0.06	12.38 ± 0.51
Pax	white	5.16 ± 0.24	9.47 ± 0.78	3.17 ± 0.23	17.80 ± 1.25
Biała Dama	white	3.15 ± 0.29	2.38 ± 0.26	2.63 ± 0.31	8.16 ± 0.86
White Virgin	white	5.43 ± 0.31	5.91 ± 0.35	8.24 ± 0.72	19.58 ± 1.39
Diana	white	2.35 ± 0.18	1.09 ± 0.08	1.34 ± 0.05	4.78 ± 0.31
Profesor Wóycicki	yellow	6.01 ± 0.13	3.60 ± 0.16	5.13 ± 0.11	14.74 ± 0.39
Calypso	yellow	4.99 ± 0.27	2.52 ± 0.13	6.68 ± 0.12	14.19 ± 0.52

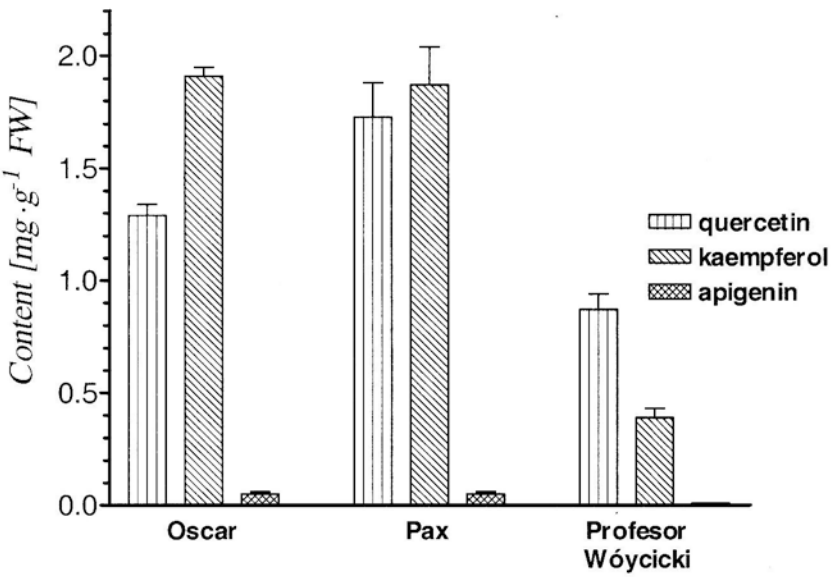

Fig. 1. Content of flavonoids in white perianth of cvs. Oscar and Pax, and in yellow perianth of cv. Profesor Wóycicki

The flavonoids are present only as glycosides. In our preliminary studies we have not found in tulip tissues measurable quantities of quercetin, kaempferol and apigenin as a underivatized aglycones (data not shown). Unexpectedly, perianths of white tulip cultivars (Oscar and Pax) contained much more glycosides of quercetin and kaempferol than perianths of yellow cultivar – Profesor Wóycicki. It seems like flavonoid glycosides occur there have white colour, or pH of tissue caused such colour. Colour of perianths in cv. Profesor Wóycicki tulips is affected by presence of carotenoids, probably. S h i b a t a and I s h i k u r a (1960) showed that yellow cultivars of tulips contained kaempferol and quercetin glycosides, and violoxanthin as main component. Red, orange, pink and purple perianths of tulip contains glycosides of cyanidin, pelargonidin and delphinidin, carotenoids and flavonol glycosides: quercetin, kaempferol and myricetin (S h i b a t a and I s h i k u r a, 1960).

Anthers of all studied tulip cultivars had yellow colour. Concentration of all investigated flavonoids was much higher than in perianths (Table 1). Anthers contained glycosides of apigenin, quercetin and kaempferol in similar contents. Lowest amounts of flavonoids were found in anthers of cv. Biała Dama and cv. Diana (with white perianths). Total concentration of flavonoids ranged from 4.78 mg · g^{-1} (cv. Diana) up to 19.57 mg · g^{-1} FW (cv. White Virgin). The level of flavonoid glycosides in tulip anthers is exceptionally high, and is comparable to that found in dry scales of onion (10 - 30 mg · g^{-1} DW). According to results published by S t r a c k et al. (1981) pollen of tulip cv. Apeldoorn contained several glycosides of quercetin, kaempferol and isorhamnetin. Colour of anthers in tulip cv. Apeldoorn is violet. In our investigations we found in yellow anthers (on base of retention time) the presence of apigenin. In extract from tulip anthers fortified with known amounts of standard, height and area of apigenin peak increased proportionally. It is possible that yellow anthers contain apigenin, although in the violet anthers and pollen isorhamnetin was found. For proving of the presence in tulip anthers of apigenin glycosides further studies are planned.

V a l a d o n and M u m m e r y (1968) have shown the occurrence of many carotenoids in yellow perianths, yellow anthers, yellow stigmas and deep orange filaments of tulip cv. Golden Harvest. However our studies carried out on yellow and white tulip perianths and yellow anthers, have indicated that its colour may be affected by presence of different flavonoids too.

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Flawonoidy w białym i żółtym okwiecie i żółtych pylnikach tulipanów *(Tulipa gesneriana* L.)

Streszczenie

Analizowano zawartość flawonoidów (metodą HPLC) w białym i żółtym okwiecie i w żółtych pylnikach kilku odmian tulipanów w okresie pełni kwitnienia. W żółtych pylnikach analizowanych odmian (Oscar, Pax, Profesor Wóycicki, Biała Dama, White Virgin, Calypso, Diana) wykazano wysoką zawartość kwercetyny (2,35 - 6,01 mg · g^{-1} ś.m.), kempferolu (1,09 - 9,47 mg · g^{-1} ś.m.) i apigeniny (1,34 - 8,24 mg · g^{-1} ś.m.). W białym okwiecie odmian Oscar i White Virgin stwierdzono również wysoki poziom kwercetyny (1,30 - 1,80 mg · g^{-1} ś.m.) i kempferolu (1,90 mg · g^{-1} ś.m.), a tylko ilości śladowe apigeniny. W żółtym okwiecie odmiany Profesor Wóycicki zawartość kwercetyny i kempferolu była dużo mniejsza niż w białym okwiecie odmian Oscar i White Virgin, a brak było apigeniny. Tak więc żółte pylniki i biały i żółty okwiat odmian tulipanów są bogatym źródłem flawonoidów.