Changes in Endogenous Flavonoids Level during Cold Storage of Tulip Bulbs

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Abstract

Although much attention has been paid to the physiological processes associated with dormancy release during cold treatment of tulip bulbs, all analyzed metabolic processes have thus far not been sufficient criteria for measuring efficient low temperature treatment. The aim of this work was to analyze changes of endogenous flavonoids levels in leaves and anthers during cooling of tulip bulbs. The flavonoids are present as glycosides in leaves and anthers. During bulb cooling the leaf content of quercetin and kaempferol (after hydrolysis) substantially increased (in comparison to uncooled bulbs stored at 17°C). The anther content of quercetin and apigenin greatly increased during storage of bulbs at high temperature and was low in cooled bulbs. The level of kaempferol in anthers was higher in cooled than in uncooled bulbs. The possible role of these changes in dormancy release is discussed.

INTRODUCTION

During the development of tulip flowers three phases can be distinquished (Boonekamp et al., 1990): (i) the initiation and formation of a new sprout with flower (at high temperature); (ii) the internal preparation for stem elongation (at low temperature); (iii) the rapid elongation of the sprout (at high temperature). Tulip bulbs, with a terminal bud containing a complete flower, require 12-16 weeks of low temperature treatment for floral stalk elongation. This suggests a kind of dormancy that can be released by exposure to low temperature (Kamerbeek et al., 1972). The duration of the cold treatment is a major factor determining stalk growth and flowering. Increase of duration of low temperature treatment decreases the number of days from planting to flowering. Stem and leaf extension of cooled tulip bulbs is due almost entirely to the elongation of cells produced early in the development of the flower bud (Gilford and Rees, 1973).

Considerable attention has been paid to hormonal control of dormancy induction, in dormancy release and in shoot growth after tulip bulb cooling (Saniewski et al., 2000). The induction of flowering and growth of the floral parts in tulip bulbs during cold treatment has also been studied by several groups. These studies concerned processes such as changes in various carbohydrate levels and the activities of α-amylase and invertase (Lambrechts et al., 1992b, 1994; Gorin and Heidema, 1985; Heidema et al., 1986; Ohyama et al., 1988; Lambrechts and Kollöffel, 1993; Balk and de Boer, 1999), the amino acid composition (Łukaszewska et al., 1989; Tonecki and Gorin, 1990; Lambrechts et al., 1992a), and the presence of chalcones and the activity of chalcone synthase (Sutfeld and Wiermann, 1980; Gorin et al., 1990; Franssen and Kersten, 1992). All these processes turned out to be no good criteria for measuring efficient duration of cold treatment. We therefore analysed another parameter.

Flavonoids are a large group of naturally occurring compounds of particular importance as they are have been found to posses antioxidant and free radical scavenging activity (Iwashina, 2000; Pietta, 2000). Flavonoids apparently important in defense against pathogens and insects and protect plants against stress conditions during growth and development (Harborne, 1994). For example, flavonoids protect plants against free radicals and oxidative damages caused by UV radiation in sunlight (Larson, 1988). Flavonoids apparently play a role in auxin biosynthesis and are responsible for colour and

taste of several plant tissues (Moore, 1989).

In tulip organs various flavonols have been (Strack et al., 1981; Budzianowski, 1991). Most literature on tulips concerns identification and measurements of anthocyanidins (in the perianth, anthers and pollen; Nakayama et al., 1999; Nieuwhof et al., 1990; van Raamsdonk, 1993; Halevy and Asen, 1959; Shibata and Ishikura, 1959, 1960; Shibata and Yoshitama, 1968; Shibata and Sakai, 1961; Torskangerpoll et al., 1999). The aim of this work was to study the levels of some flavonoids during dormancy and dormancy release of tulip bulbs.

MATERIAL AND METHODS

Levels of the flavonoids quercetin and kaempferol were determined in leaves and anthers excised from tulip (*Tulipa gesneriana* L.) bulbs cv. Apeldoorn. Bulbs (with a circumference of 12-14 cm) were sampled both during cooling at 5^{o}C and during storage at 17^{o}C. Analysis started October 15 and continued until the end of January, at two week intervals.

The flavonoid content was determined according to a method based on Patil et al. (1995) with several modifications (Horbowicz, 1999). Thirty bulbs were taken for each analysis. After cutting a 20 g sample of leaf tissue, the material was blended with a 10-fold (v/w) of 60% ethanol-water. Anthers (0.5 g samples) were homogenized with a 20-fold volume of 60% ethanol-water. Samples were kept overnight at ambient temperature, and then filtered over medium speed filter paper. 0.5 ml 6N HCl was added to 1.5 ml aliquots placed in screw-capped vials, and flavonoid glycosydes were hydrolysed at 100^{o}C for 30 min. One ml of water was added to the hydrolysate and the aglycones obtained were extracted by three times vigorous shaking with 1 ml of ethyl acetate. The separated upper layer was withdrawn using a Pasteur pipette. Pooled acetate layers were diluted with methanol: water solution (1:1, v/v).

We used 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 a Shimadzu C-R6A Chromatopac integrator. The flavonols were isocratically separated on Lichrosorb RP18 (4 x 250 mm, 10 µm) column. The mobile phase was a methanol: water mixture (55:45, v/v) that contained 0.2% ortho-phosphoric acid. The flow rate was 0.8 ml/min.

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

Analyses were made in three biological replicate samples. Results were statistically analysed using the Student-t test.

RESULTS AND DISCUSSION

The flower buds, the leaves, perianth, stem and anthers were smaller in fully cooled tulip bulbs (5^{o}C) than in uncooled bulbs kept continuously at 17^{o}C (Fig. 1 and 2). The growth and development of tulip flowers, after planting uncooled and cooled bulbs is shown in Fig. 3.

The flavonoids tested were not detected in dry and fleshly scales, stem, perianth and pistil, both in uncooled and cooled tulip bulbs, but were present in leaves and anthers. The flavonoids were present only as glycosides. We did not find measurable quantities of quercetin and kaempferol as underivatized aglycones.

During cool storage the content of quercetin and kaempferol substantially increased in the leaves. Levels of these compounds were much lower and did not increase in the leaves in uncooled bulbs (Fig. 4). The content of quercetin and apigenin in the anthers substantially increased during storage of the bulbs at high temperature (17^{o}C), whereas levels remained low in cooled bulbs (Fig. 5). The level of kaempferol in the anthers was substantially higher in cooled than in uncooled bulbs (Fig. 5).

We here present data of experiments made in 2002/2003. Similar results were obtained in 2000/2001 and 2001/2002.

The role of the changes in endogenous flavonoids levels, if any, is unknown. Flavonoids, mainly quercetin and kaempferol, are endogenous auxin transport inhibitors (Jacobs and Rubery, 1988; Dakora, 1995; Murphy et al., 2000), inhibitors of lipid peroxidation (Whittern et al., 1984; Takahama, 1985; Alcaraz et al., 1986; Torel et al., 1986), strong antioxidants (Harborne, 1994; Formica and Regelson, 1995; Pietta, 2000) and substrates for peroxidase (Takahama and Oniki, 2000).

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Figures

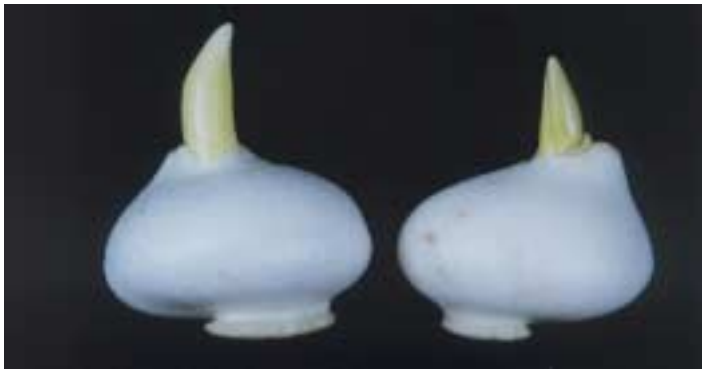

Fig. 1. Tulip bulb without cooling (left) and after full cooling (right); photographed February 2.

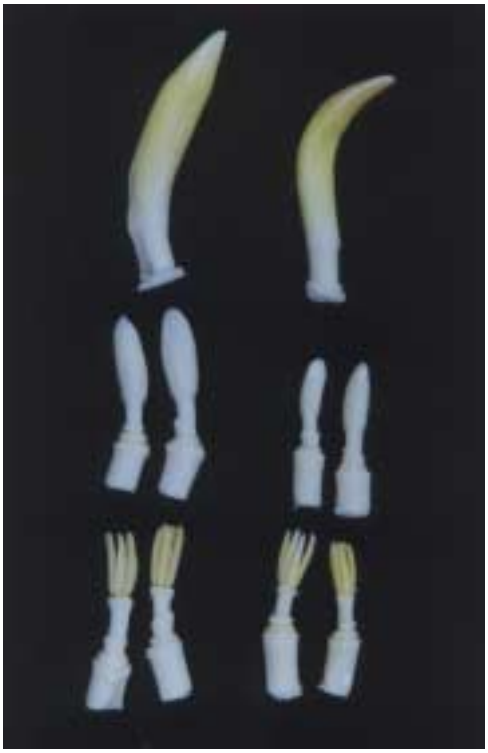

Fig. 2. Flower bud isolated from uncooled tulip bulb (on left) and after cooling (right); photographed February 2.

Fig. 3. Growth and development of tulip after planting of uncooled bulb (left) and fully cooled bulb (right); photographed February 27.

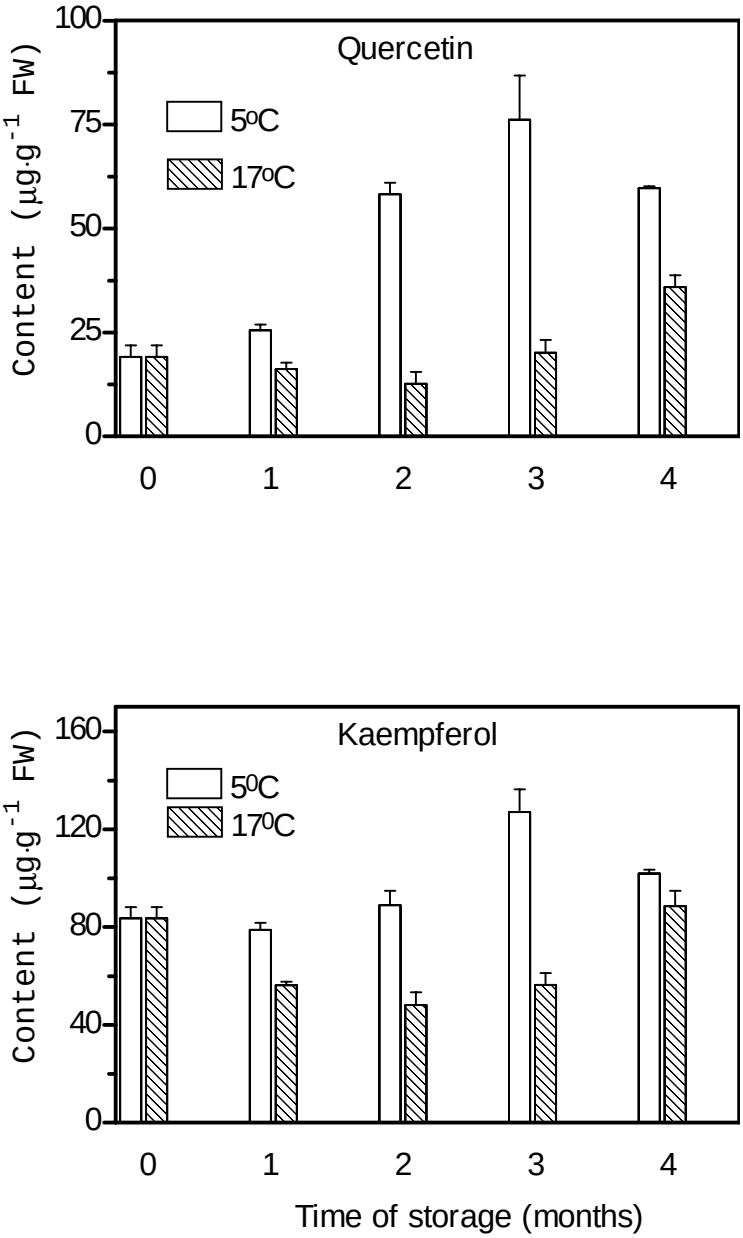

Fig. 4. The content of quercetin and kaempferol in leaves isolated from uncooled tulip bulbs (17°C) and from bulbs after different period of cooling (5°C).

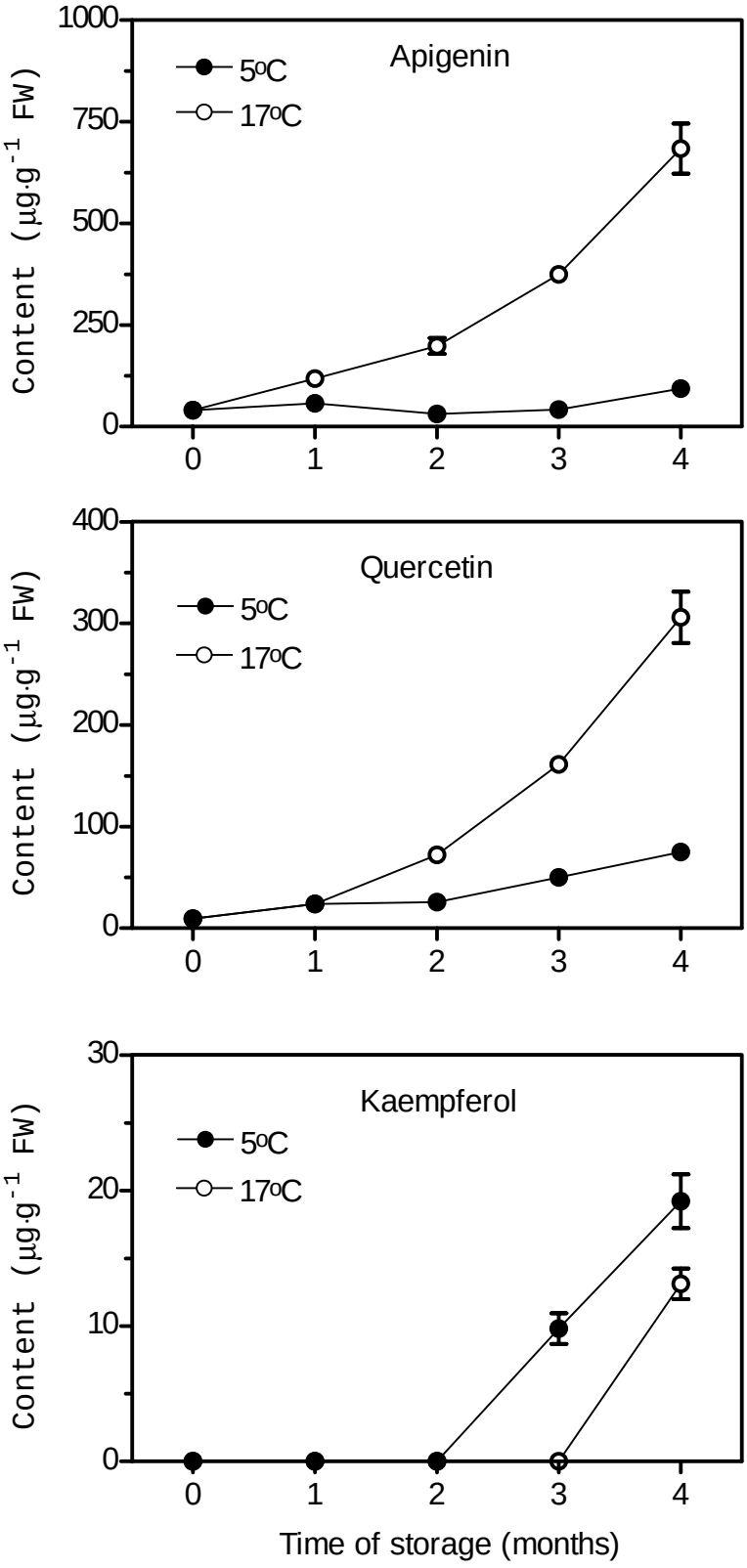

Fig. 5. The content of quercetin, kaempferol and apigenin in anthers isolated from uncooled tulip bulbs (17°C) and from bulbs after different period of cooling (5°C).