Only Full Cooling of Mother Tulip Bulbs Is Necessary for Daughter Bulb Formation from Daughter Buds

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Keywords: tulip, *Tulipa gesneriana*, cooling, mother bulbs, daughter bulb formation, daughter buds

Abstract
Mother tulip bulbs planted in the autumn require a long cold period before satisfactory stem extension with flower development and enlargement of daughter buds to bulbs will occur in spring. Little is known of the underlying physiological mechanism of daughter bulb formation from daughter buds. In our studies we used tulip bulbs cv. Apeldoorn cooled at 5°C. We found that for the growth of daughter bulbs in tulips, only full cooling of mother bulbs was necessary, not the growth of shoot and roots when the bulbs were stored at room temperature after cooling (without planting of bulbs). It was interesting that daughter bulbs were formed when mother bulbs are stored for extended periods (about seven months) at 5°C. In most of the daughter bulbs that formed in these conditions, flower bud differentiation took place and flowering occurred during the next season after sufficient cooling. The mechanisms of transporting food reserves from the mother bulb directly to daughter buds to grow daughter bulbs, including the role of plant hormones and flavonoids, is discussed.

INTRODUCTION
During the development of tulip flowers, three phases can be distinguished (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); and (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. Increasing the duration of low temperature treatment decreases the number of days from planting to flowering.

A low temperature treatment of tulip bulbs simultaneously induces not only a stimulation of shoot growth but also the bulbing of the axillary buds; a faster plant elongation is also associated with a more rapid bulbing of the axillary buds (Le Nard and De Hertogh, 1993). Thus, extending the duration of the cold treatment results in a very rapid expression of bulbing (Aoba, 1976; Le Nard and De Hertogh, 1993).

Daughter buds are located at the inner bases of the scales, and generally there is one bud per scale. The transformation of the daughter buds into daughter bulbs is especially rapid after flowering and simultaneously, the mother-bulb scales shrivel and progressively disappear (Le Nard and De Hertogh, 1993). The innermost bud produces the largest bulb, while the other buds produce smaller bulbs with fewer scales. At the end of spring, daughter-bulb enlargement ceases.

The aim of the present work was to study the formation of daughter bulbs in fully cooled mother tulip bulbs stored at room temperature without planting, including excision of sprouts and root primordia, under light and darkness conditions.
MATERIAL AND METHODS
Tulip bulbs cv. Apeldoorn, with circumference of 10-11 cm, were stored at 17-20°C after lifting until October 15 and then were dry-cooled at 5°C until the end of July or until used in February-March. Other tulip bulbs were stored at the same time at 17°C as uncooled bulbs. Fully cooled and uncooled bulbs were stored at room temperature (20-23°C) without rooting as intact bulbs after excision of sprouts and roots, under light and darkness conditions. Morphological observation of daughter bulb formation and measurement of their weight were made in cooled and uncooled mother bulbs. A total of 20 to 25 bulbs were used per treatment and experiments were repeated during two growing seasons.

RESULTS AND DISCUSSION
At planting, dormant tulip bulbs are composed of fleshy scales attached to a basal plate that produces roots at the basal surface, differentiated apical flower bud (stem, leaves and all parts of flower), central daughter bud, other axillary buds an “H” bud, and tunic (dry outer bulb scale).

The size of flower bud and of the particular organs (leaves, perianth, stem, and anthers, and daughter buds) in fully cooled tulip bulbs (at 5°C) was a little smaller than in uncooled bulbs continuously at 17°C (Fig. 1). The growth and development of tulips after planting of uncooled and cooled bulbs is presented in Fig. 2. Natural shoot growth and flowering only occurred from fully cooled bulbs.

In fully cooled tulip bulbs stored at room temperature under natural light, enlargement of daughter bulbs took place, whereas no enlargement occurred in uncooled mother bulbs stored under the same conditions (Fig. 3, Table 1). Excision of sprouts and/or roots primordia from fully cooled tulip mother bulbs did not prevent daughter bulb growth at room temperature (Fig. 4, Table 1). It was interesting that daughter bulbs were also formed when mother bulbs were continuously stored for extended periods (about seven months) at 5°C (Fig. 5). In most of the larger size daughter bulbs that formed in cooled mother bulbs stored at room temperature, flower buds developed and flowering took place in the field during the following season (Fig. 6).

Translocation of both carbohydrates and inorganic nutrients from mother-bulbs to the daughter bulblets is well documented (Schmalfeld and Carolus, 1965; Aung et al., 1973). Aung et al. (1973) found that during the ontogeny of cv. Preludium bulblets in the field, the decrease in soluble sugars and starch contents of the mother-bulbs scales corresponded with a concomitant increase of these carbohydrates in the bulblets. They noted also that while all the soluble sugars in the matured bulblets were accounted for by the initial sugars of the mother bulbs scales, only 17% of the bulblet starch could be accounted for by the initial starch content of the bulbs, with the remaining 83% of the starch being derived from photosynthates.

Limited information is available regarding hormonal control of daughter bulb development in field-grown tulips. Aung and Rees (1974) determined the changes in the endogenous gibberellins content of the developing bulblets derived from field-grown bulbs cv. Apeldoorn. They showed that the gibberellin activity of bulblets increased dramatically in November-March and declined sharply in April-July. The increase in gibberellin-like substances was considered to be derived primarily by synthesis within the bulblets with possible contributions via translocation from the mother bulb scales, roots, and shoots.

Recently, Saniewski and Horbowicz (2004) showed the occurrence of quercetin and kaempferol as glycosides in leaves and anthers of uncooled and cooled tulip bulbs. During cold storage of tulip bulbs, the content of quercetin and kaempferol substantially increased in the leaves and the level of these compounds was much lower and stable in leaves of uncooled bulbs. In the case of anthers, the content of quercetin and apigenin greatly increased during storage of bulbs at high temperature (17°C) and was low in cooled bulbs. The level of kaempferol in anthers was substantially higher in cooled than uncooled bulbs.
These flavonoids were not detected in dry and fleshly scales, stem, perianth, and pistil in both uncooled and cooled tulip bulbs.

The role of these changes in endogenous flavonoid levels in leaves and anthers during cooling of tulip bulbs in dormancy release and daughter bulb formation is unknown. Flavonoids, mainly quercetin and kaempferol, as 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 (Pietta, 2000), and substrates for peroxidase (Takahama and Oniki, 1997, 2000), may play an important role in many physiological processes, including dormancy release and daughter-bulb enlargement in mother tulip bulbs as a result of low temperature treatment.

**ACKNOWLEDGEMENT**

This work was partially supported by a Grant No 6/P06A/011/21 from State Committee for Scientific Research (Poland).

**Literature Cited**


Table 1. The effect of removal of sprouts and root primordia in fully cooled mother tulip bulbs and their storage in light or darkness at room temperature on daughter bulb weight.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage conditions: darkness (D) or light (L)</th>
<th>Weight of daughter bulbs (g)</th>
<th>Outermost axillary bud</th>
<th>Innermost daughter bulbs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>A</td>
</tr>
<tr>
<td>Intact bulbs</td>
<td>L</td>
<td></td>
<td>0.7</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td></td>
<td>0.7</td>
<td>7.6</td>
</tr>
<tr>
<td>Excised sprouts</td>
<td>L</td>
<td></td>
<td>0.9</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td></td>
<td>0.9</td>
<td>6.2</td>
</tr>
<tr>
<td>Excised sprouts and root primordia</td>
<td>L</td>
<td></td>
<td>0.7</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td></td>
<td>0.6</td>
<td>4.1</td>
</tr>
</tbody>
</table>
Fig. 1. Tulip bulbs without cooling – stored at 17°C (on left) and cooled – stored at 5°C (on right); photographed on March 15. A – intact bulbs, B – isolated flower bud and daughter buds.
Fig. 2. Growth and development of tulips after planting of uncooled (on left) and fully cooled bulbs (on right) (see Fig. 1.)

Fig. 3. Uncooled and cooled tulip bulbs (see Fig. 1) were kept for one month at room temperature (20 to 23°C) in natural light conditions. A – intact bulbs, uncooled and cooled, respectively, B – daughter buds and daughter bulbs, isolated from uncooled and cooled mother bulbs, respectively.
Fig. 4. Cooled tulip bulbs (see Fig. 1) were kept for one month at room temperature (20 to 23°C) in natural light conditions after the following treatments: On left – intact mother bulb, in middle – removed upper part of sprout, on right – removed upper part of sprouts and root primordial. In all cases, outermost axillary bulb and inner daughter bulbs are formed.

Fig. 5. Tulip bulbs were stored at 5°C from October 15 until the end of August; long storage of mother bulbs at low temperature causes formation of outermost axillary bulb and inner daughter bulbs.
Fig. 6. Daughter bulbs formed in fully cooled mother bulbs after storage for one month at room temperature (A) and flowering of these daughter bulbs under field conditions (B).