Susceptibility and/or Responsiveness of Tulip Stem Segments Excised from Cooled or Uncooled Bulbs to Indole-3-Acetic Acid

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ABSTRACT

The growth of stem segments excised from cooled and uncooled tulip (Tulipa gesneriana L. ‘Apeldoorn’) bulbs stored for three months after flower bud formation at 5°C and 17°C, respectively, was enhanced by indole-3-acetic acid (IAA) at 0.1% (w/w) in the light and in dark conditions, when it was applied to the place where flower buds were removed. On October 27, the enhanced growth of excised stem segments induced by IAA was very reduced under natural light conditions. On the other hand, the susceptibility and/or responsiveness of tulip stem segments excised from cooled or uncooled bulbs to exogenously applied IAA increased as the age or maturation of tulip bulbs increased until January 15 of the following year. The ability of tulip stem segments to elongate did not depend on low temperature and light conditions. A possible explanation for IAA-induced growth of tulip stem segments is discussed from the aspect of the solutes in the cell sap and on the balance of endogenous plant hormones in the bulbs.

Keywords: aging, auxin, cold treatment, light condition, maturation, Tulipa gesneriana

INTRODUCTION

Tulip bulbs with terminal buds containing a complete flower require a period of 12-16 weeks of cold (low temperature) treatment for floral stalk elongation, indicating that tulip bulbs have a kind of dormancy released by exposure to low temperature (Kamerbeek et al. 1972). The duration of the cold treatment is a major factor that determines how the stem grows and how the flower opens. Increasing the duration of cold treatment decreases the time from planting to flowering. Enlargement of the stem and leaves of cooled tulip bulbs is almost entirely due to the elongation of cells produced early in the development of the flower bud (Gilfedder and Rees 1973).

Auxins are well known to play an important role in the growth and development of tulips. The leaves and gynoeicum have been suggested to provide auxins for inducing stem growth (Op den Kelder et al. 1971; Hanks and Rees 1977; Saniewski and de Munk 1981; Banasik and Saniewski 1985). Excision of all leaves and the flower bud in the early growth stage of tulips resulted in total inhibition of stem growth. The exogenous application of auxin such as indole-3-acetic acid (IAA, at 0.005–0.1%, w/w in lanolin), naphthaleneacetic acid (NAA, at 0.05–0.2%, w/w in lanolin) and indole-3-butyric acid (IBA, at 0.2%, w/w in lanolin) to the place where the flower bud had been removed almost completely recovered stem growth of tulips (‘Apeldoorn’ and ‘Oxford’) (Saniewski and de Munk 1981; Banasik and Saniewski 1985). Removal of IAA applied exogenously in different growth stages of tulip stem immediately stopped further elongation of each internode, indicating that continuous supply of IAA is necessary for tulip stem growth (Saniewski and Węgrzynowicz-Lesiak 1993).

IAA applied at the cut surface of the stem with all leaves and the flower bud removed also induced stem growth in uncooled-rooted and -derooted tulip bulbs (‘Apeldoorn’ and ‘Gudoshnik’), but the effect of auxin was much smaller than in cooled bulbs (Saniewski and Okubo 1997). Recently, Saniewski et al. (2005a) showed that the application of IAA as a lanolin paste at the cut surface of the top internode in stem segments prepared from growing shoots of cooled tulip bulbs (‘Apeldoorn’) greatly promoted the growth of all internodes of the stem. These results described above strongly suggest that the promoting-effect of IAA on inducing growth of intact and/or excised stem of tulips is affected by the culture (experimental and application) conditions. In addition, it has been shown that solutes such as carbohydrates, including starch, sucrose, glucose and fructose, found at the first internode were responsive to promote the elongation of the upper internodes induced by auxin (Saniewski et al. 2005a). Susceptibility and/or responsiveness of tulip stem segments excised from cooled or uncooled bulbs to IAA, however, have not been clarified yet. In a strenuous effort to understand the susceptibility and/or responsiveness of stem segments of tulips (‘Apeldoorn’) to IAA applied exogenously, we successfully discovered a novel fact: the important factor for susceptibility and/or responsiveness to IAA is an increase in the age or maturation of tulip bulbs but not temperature and light conditions. In this paper we report this novel finding and also propose the possible mechanisms to regulate this responsiveness based on aspects of solutes in the cell sap and on the balance of endogenous plant hormones.

MATERIALS AND METHODS

Tulip (Tulipa gesneriana L. ‘Apeldoorn’) bulbs with a circumference of 10-11 cm were obtained from a private farm in Poland. After lifting at the end of June, tulip bulbs were stored at 17-20°C until October 27, and then at 5°C (cooled bulbs) or at 17°C (uncooled bulbs) until January 15. Stem segments consisting of the 1st internode in stem segments prepared from growing shoots of Tulipa gesneriana L. ‘Apeldoorn’ and ‘Oxford’ (Saniewski and de Munk 1981; Banasik and Saniewski 1985). Removal of IAA applied exogenously in different growth stages of tulip stem immediately stopped further elongation of each internode, indicating that continuous supply of IAA is necessary for tulip stem growth (Saniewski and Węgrzynowicz-Lesiak 1993).

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or without IAA (Fluka, Switzerland) at 0.1% (w/w) was applied at the cut surface, the place where the flower bud was removed, of the stem segments. The stem segments were incubated with water at 18-20°C under natural light conditions in the greenhouse or under dark conditions in a phytotron, then the length of stem or different internodes was measured. Ten to 15 stem segments excised from tulip bulbs were used for each treatment. At the end of the experiments, the stem segments were photographed. Data were subjected to an analysis of variance and evaluated using the Duncan’s multiple range test at a 5% level of significance.

RESULTS AND DISCUSSION

The flower buds, leaves, perianth, anthers and stem in fully cooled (5°C) tulip bulbs (‘Apeldoorn’) were slightly smaller than those from uncooled bulbs kept continuously at 17°C (Saniewski and Horbowicz 2005). Only after planting could the complete differences in growth and development between cooled and uncooled tulip bulbs be found to be normal with growth of the shoot and flowering being observed in fully cooled bulbs (‘Apeldoorn’) (Saniewski and Horbowicz 2005).

The induction of growth by IAA in excised stems on October 27 under natural light conditions was very small (Fig. 1), suggesting that the segments were almost unsusceptible and/or unresponsive to IAA applied exogenously. This result is similar to that in the case of gibberellin A 3 (GA3 at 200 mg/l) in tulips (‘Oxford’) (Geng et al. 2005). On the other hand, IAA-induced growth of stem segments excised from uncooled bulbs and from partially cooled bulbs on November 23 was significantly evident under natural light conditions (Fig. 2, 3). Furthermore, while the growth of stem segments prepared from uncooled and fully cooled tulip bulbs on January 15 treated with lanolin only and kept in natural light conditions was very small (Figs. 4-6), the application of IAA at the cut surface of the stems described above greatly enhanced stem growth in natural light conditions (Figs. 4, 6). A similar effect of IAA on stem growth was also observed in the dark (Figs. 5, 6). The stimulatory effect of IAA on stem growth was more than ca. 200 to 300% regardless of the cold treatment and light conditions; the highest stimulatory effect of IAA on stem growth was observed in excised stems from uncooled bulbs incubated under natural light conditions (Fig. 6). These results strongly suggest that the important factor for susceptibility and/or responsiveness of tulip stem segments excised from cooled and uncooled bulbs to IAA exogenously applied is the age or maturation of bulbs rather than temperature and light conditions. These results described above together with the previous reports in ‘Apeldoorn’ (Saniewski et al. 2005a) and ‘Oxford’ (Geng et al. 2007) tulips strongly support the idea that the quality and/or the quantity of storage materials such as carbohydrates in the tulip bulbs are affected during storage, and that IAA as well as GAs substan-

tially affects the metabolism of storage materials by utilizing the solutes of cell sap to induce cell elongation in tulip stems.

The idea described above might answer the following two important questions: (1) Why does IAA not induce growth of excised stems on October 27 but greatly induce growth on January 15 in uncooled and cooled bulbs? (2) Why does growth of excised stems prepared from uncooled bulbs on January 15 have almost the same susceptibility to IAA as stems from cooled bulbs? A possible answer to the
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The first question might be that after flower bud formation in tulip bulbs in October one or more processes may be connected with the ability (sensitivity) of stem cells to elongate both in low (cooled) and high (uncooled) temperatures in January.

In addition, the following two important questions are also raised from this study: (3) Why is the elongation growth of stem segments prepared from cooled and un-cooled bulbs induced by IAA almost similar in darkness and in the light? (4) Why do the normal growth of shoots and flowering take place only in fully cooled tulip bulbs?

The fact that sufficient growth of the tulip stem is observed in fully cooled bulbs but not in uncooled bulbs (Kamerbeek et al. 1972) strongly supports the idea that adequate levels of endogenous auxins to induce stem growth are produced only in fully cooled bulbs (Saniewski and de Munk 1981). However, the ability of stem cells within tulip shoots inside the bulbs become susceptible and/or responsive to exogenously applied auxin not only in fully cooled tulip bulbs but also in uncooled or partially-cooled tulip bulbs (‘Apeldoorn’) (Figs. 2-6). In addition, this ability seems to be dependent on an increase of the age or maturation of bulbs. This is the first report that shows that the important factor for susceptibility and/or responsiveness of excised tulip stem segments to auxin applied exogenously is not low temperature and/or light condition but only an increase in the age or maturation of the bulb.

The reason why stem cells in only mature tulip bulbs are susceptible and/or responsive to exogenously applied auxin is still not clearly understood. Similar seasonal differences in the response to GA treatment have also been reported in non-pre-cooled tulip bulbs (‘Oxford’) (Geng et al. 2005). One possible mechanism is the increase in the total amount of solutes in the cells as one major parameter for cell elongation. In stems excised from growing shoots of cooled tulip bulbs, exogenously applied IAA has been suggested to enhance the translocation of solutes from the basal region to the upper elongating region of shoots, and the source size or the amount of solutes is responsible for IAA-induced elongation growth (Saniewski et al. 2005a). Starch degradation occurred in the scales of tulips (‘Oxford’) during storage regardless of storage temperature, although the rate of degradation in the scale was faster in cooled bulbs than in uncooled ones (Geng et al. 2007). These facts support the notion that susceptibility and/or responsiveness of excised tulip stem segments to auxin applied exogenously is dependent on the total amount of solutes relating to

**Fig. 4** The effect of IAA on the growth of tulip stem excised from un-cooled (17°C) and fully cooled (5°C) bulbs in natural light conditions. Treatments were made on January 15 and photographed on February 5. Photographs were initial, control (lanolin only) and IAA treatment (IAA at 0.1%, w/w in lanolin), from left to right.

**Fig. 5** The effect of IAA on the growth of tulip stem excised from un-cooled (17°C) and fully cooled (5°C) bulbs in darkness. Treatments were made on January 15 and photographed on February 5. Photographs were initial, control (lanolin only) and IAA treatment (IAA at 0.1%, w/w in lanolin), from left to right.

**Fig. 6** The effect of IAA on the growth of tulip stem excised from uncooled (17°C) and fully cooled (5°C) bulbs in the dark or natural light conditions. Treatments were made on January 15. Length of stem segments was determined from January 26 to February 1. Different letters indicate mean separation on each date of measurements for all four treatments, uncooled-dark, uncooled-light, cooled-dark and cooled-light, by the Duncan’s multiple range test at 5% significance. Bars = SE (n=10-15).
endogenous changes in metabolism such as source activity during aging, or maturation of the bulb.

As shown in Figs. 4-6, regardless of the light conditions exogenously applied IAA greatly promoted the growth of segments excised from cooled and uncooled tulip bulbs (‘Apeldoorn’). In addition, the responsiveness to IAA was little affected by natural light or dark conditions. We also found that the effect of IAA on the growth of excised stems is less than that in the dark and that of excised stems from cooled dark and uncooled tulip bulbs (H. Okubo, K. Miyamoto and J. Ueda, unpublished results). After planting, however, the growth of shoots in intact fully cooled tulip bulbs in darkness has been shown to be much higher than that in the light conditions (Okubo and Uemoto 1984; M. Saniewski, H. Okubo, K. Miyamoto and J. Ueda, unpublished results). This discrepancy between intact tulip shoots and its stem segments might be explained by the difference in the total amount of solutes contributing to IAA-inducing cell elongation in the dark conditions. The growth of excised stems prepared from cooled and uncooled tulip bulbs is probably regulated by the availability of free sugars (and other compounds) that exist in the intact stem. It is probable that not only the endogenous levels of sugars but also their availability are similar in excised stems from cooled and uncooled tulip bulbs. This is intricately related to carbon and nitrogen metabolism in the tulip bulbs (Oh-yama et al. 2006).

Another possible mechanism related to the decrease and/or increase in levels of inhibitors and/or promoters in mature bulbs, respectively, has been proposed. Plant growth and development are regulated by the endogenous balance between promoters such as auxin and GA, and inhibitors such as abscisic acid (ABA). This relationship is also true in tulip shoot growth. Saniewski et al. (1999) have shown that simultaneous application of GA with ABA totally inhibited shoot growth and flowering induced by GA in uncooled, derooted tulip bulbs (‘Apeldoorn’). Recently, ABA content in the scales of tulips (‘Oxford’) gradually decreased as the duration of storage was extended regardless of storage temperature, suggesting that the time-dependent decrease in duration of storage was extended regardless of storage temperature, of GA1, GA4, GA9, GA12, GA24 and GA34 have been identified in both cooled and uncooled tulip bulb sprouts (‘Apeldoorn’). (Rebers 1992; Rebers et al. 1994, 1995). Among them, GA4 seemed to be the major GA, and GA12, GA6 and GA24 were also detected in lower amounts. Rebers et al. (1995) suggested that there was no direct correlation between cold-stimulated growth and a change in endogenous GA status in sprouts or basal plates of tulip bulbs during cold storage. Rebers et al. (1995) also reported that the higher level of GA4 and its inactivation product GA12 were observed only in the floral stems of cooled tulip bulbs (‘Apeldoorn’), and the absence of a significant quantitative accumulation of GA1. Based on these results, they suggested that GA4 is probably involved in floral stem elongation in tulip. Saniewski et al. (1990) found that GA4 and GA1+7 applied in soaked cotton wick wrapped around all the internodes of stem, after excision of all leaves and the flower bud, obviously induced stem elongation in tulips (‘Apeldoorn’). GAAs applied as lanolin paste to the place of the flower bud removed after excision of all leaves or as soil drench did not induce stem growth of tulips. These results indicate that the growth of tulip stem induced by GA is less than that by IAA applied to the cut surface of the stem just after flower bud removal.

In addition, exogenous GA incorporated in stem tissues seems to contribute to induce the elongation of all internodes in tulips together with endogenous auxin. Auxine stimulated growth in cold storage. Rebers et al. (2003) claimed that in elongating internodes of pea (Pisum sativum L.), auxin maintains the level of the bioactive GA, GA1, by promoting GA biosynthesis and by inhibiting GA1 deactivation. Similar relationships between GAs and IAA will be possible in tulip shoots. In our preliminary experiments using tulip (‘Apeldoorn’) segments excised from cooled and uncooled bulbs, simultaneous application of GA (GA3) with IAA did not promote IAA-induced growth (M. Saniewski, H. Okubo, K. Miyamoto and J. Ueda, unpublished results).

Based on the results of this study, extensive transport of solutes in cell sap is required for maintaining adequate growth of the tulip stem. As described above, IAA might contribute to this step in tulips (Saniewski et al. 2005a). There are numerous reports in which the mode of action of GAs to promote the growth of intact plants is also connected to the translocation of solutes in cell sap (Mulligan and Patrick 1979; Aloni et al. 1986; Miyamoto and Kamasaka 1988a, 1988b, 1990; Miyamoto et al. 1992). The role of GAs in the IAA-induced growth of stem segments prepared from cooled (mature) and uncooled, and in growth and development in intact tulips under natural conditions should be intensively studied in the future. More ingenious ideas and techniques will be introduced in further studies.

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