Floriculture and Ornamental Biotechnology ©2007 Global Science Books

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

Marian Saniewski[1] • Hiroshi Okubo[2] • Kensuke Miyamoto[3,4] • Junichi Ueda[3*]

[1] Research Institute of Pomology and Floriculture, Pomologiczna 18, 96-100 Skierniewice, Poland
[2] Laboratory of Horticultural Science, Division of Agricultural Botany, Department of Plant Resources, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan
[3] Department of Biological Science, Graduate School of Science, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan
[4] Faculty of Liberal Arts and Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan

Corresponding author: * **ueda@b.s.osakafu-u.ac.jp**

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 (Gilford and Rees 1973).

Auxins are well known to play an important role in the growth and development of tulips. The leaves and gynoecium 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 to 4th internodes without flower buds, leaves and bulbs were prepared on October 27 (initial stage), November 23 (partially cooled) and January 15 (cooled), respectively. Lanolin paste with

Received: 16 August, 2007. Accepted: 19 September, 2007.

Original Research Paper

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 (GA_3 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 (**Figs. 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 substantially 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

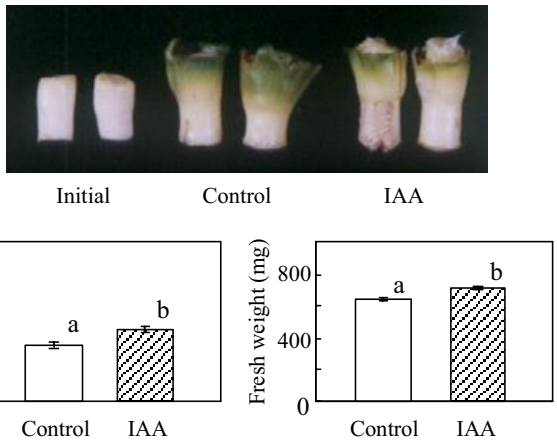

Fig. 1 The effect of IAA on the growth of tulip stem excised from uncooled bulbs; treatments were made on October 27 and photographed on November 20. Initial length and fresh weight of the stem segment were 11 mm and 321 mg, respectively. Photographs were initial, control (lanolin only) and IAA treatment (IAA at 0.1%, w/w in lanolin) from left to right. Different letters indicate the mean separation in each stem by the Duncan's multiple range test at 5% significance. Bars = SE (n=10-15).

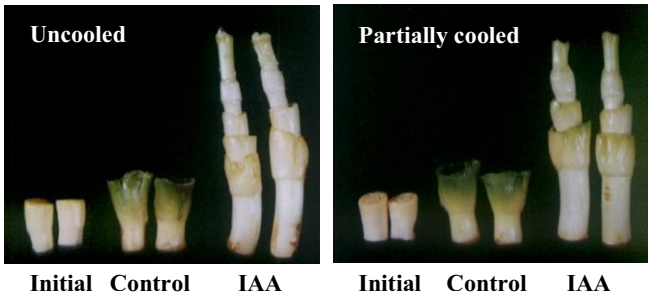

Fig. 2 The effect of IAA on the growth of tulip stem excised from uncooled (17°C) (left) and partially cooled (5°C) bulbs (right). Treatments were made on November 23 and kept in natural light conditions. Photographs were initial, control (lanolin only) and IAA treatment (IAA at 0.1%, w/w in lanolin) from left to right, and taken on December 7.

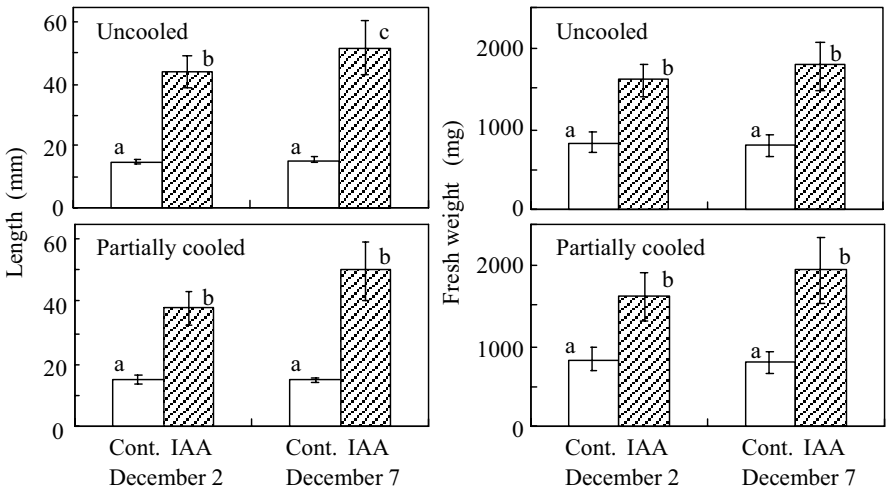

Fig. 3 The effect of IAA on the growth of tulip stem excised from uncooled (17°C) (upper) and partially cooled (5°C) bulbs (lower). Treatments were made on November 23 and kept in natural light conditions. Length and fresh weight of stem segments were determined on December 2 and 7. Different letters indicate the mean separation in each stem by the Duncan's multiple range test at 5% significance. Bars = SE (n=10-15).

Natural light conditions

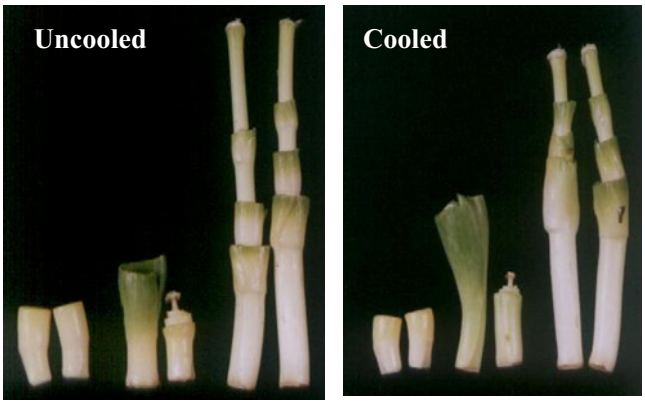

Fig. 4 The effect of IAA on the growth of tulip stem excised from uncooled (17°C) and fully cooled (5°C) bulbs in natural light conditions. Treatments were made on January 15 and photographed on February 5. Photographs were initial, control (lanolin only) and IAA treatment (IAA at 0.1%, w/w in lanolin),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

Dark conditions

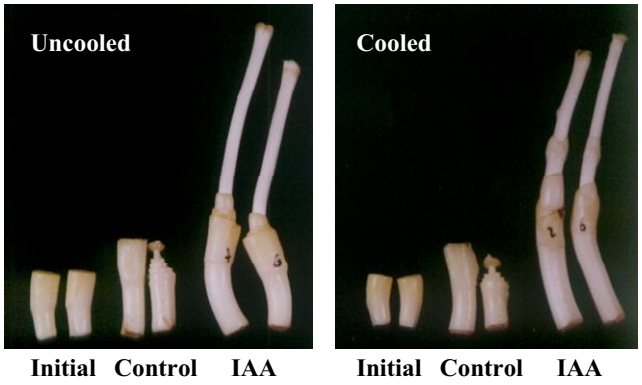

Fig. 5 The effect of IAA on the growth of tulip stem excised from uncooled (17°C) and fully cooled (5°C) bulbs in darkness. Treatments were made on January 15 and photographed on February 5. Photographs were initial, control (lanolin only) and IAA treatment (IAA at 0.1%, w/w in lanolin), from left to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

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 auxin 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 exogenously applied auxin 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

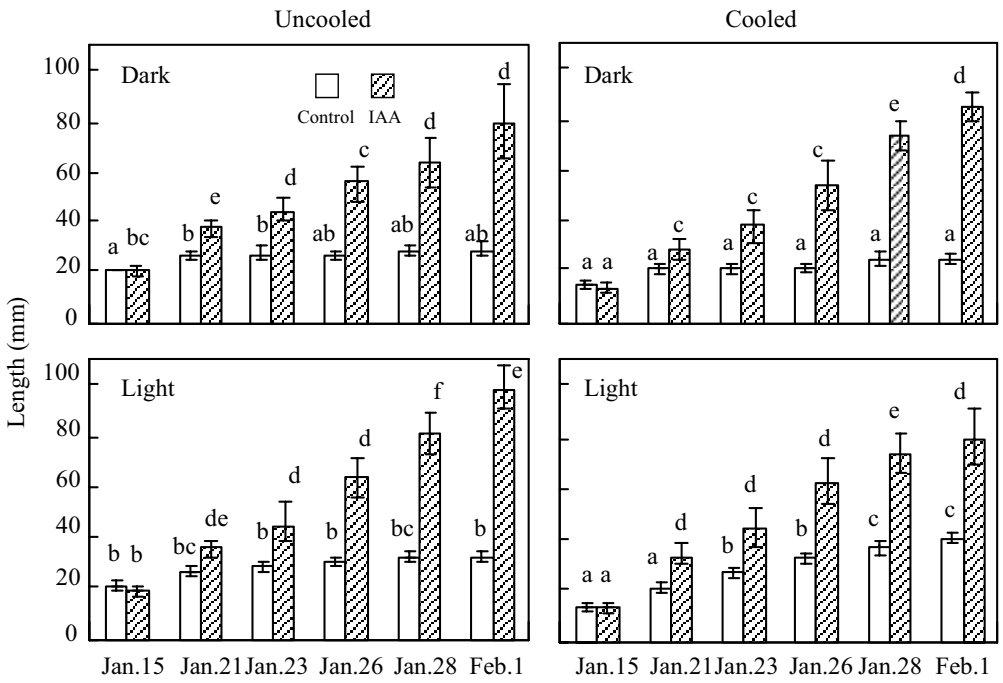

Fig. 6 The effect of IAA on the growth of tulip stem excised from uncooled (17°C) and fully cooled (5°C) bulbs in the dark or natural light conditions. Treatments were made on January 15. Length of stem segments was determined from January 26 to February 1. Different letters indicate mean separation 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 almost similar in the dark and in the light (M. Saniewski, 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 initial 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 (Ohyama *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_3 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 ABA in the scale during storage promotes sensitivity to GA after planting (Geng *et al*. 2007). As well as ABA of these tulip cultivars (Aung and Hertogh 1979; Terry *et al*. 1982; Moore 1989), jasmonates which inhibit auxin-induced growth of plants (Miyamoto *et al*. 1997; Saniewski *et al*. 2005b) have also been found in tulips as important growth regulators (Skrzypek *et al*. 2005). Therefore, it seems that excision of stems from cooled and uncooled tulip bulbs ('Apeldoorn') is connected with removal of inhibitors such as ABA and jasmonates.

The elongation of all the internodes in tulips ('Apeldoorn' and other cultivars) has been reported to be substantially controlled by the interaction of endogenous auxins and GAs (Okubo and Uemoto 1985; Okubo *et al*. 1986; Saniewski 1989; Saniewski and Kawa-Miszczak 1992; Rietveld *et al*. 2000), although the effect of GAs in the presence and/or absence of auxin on the stem growth of tulips remains controversial. Exogenously applied GAs partially substituted for the cold treatment of tulip bulbs and stimulated shoot growth and flowering (van Bragt and Zijlstra 1971; van Bragt and van Ast 1976; Rudnicki *et al*. 1976; Cocozza Talia and Stellacci 1979; Jones and Hanks 1984). Saniewski (1989) has also suggested that endogenous GAs together with auxin play an important role in stem elongation of tulips ('Gudoshnik'). According to this idea, it is possible to say that the growth of tulip stems induced by IAA applied just after removal of all leaves and the flower is due to the interaction of IAA with endogenous GAs produced in the stem. GAs or their precursors may be synthesized in the stem or transported from bulbs.

Under natural conditions GAs produced during the cooling of bulbs play an important role in the flower bud growth and those synthesized during shoot growth together with auxin control stem elongation of tulips. Free acid GAs of GA_1, GA_4, GA_9, GA_{12}, GA_{24} and GA_{34} have been identified in both cooled and uncooled tulip bulb sprouts ('Apeldoorn') (Rebers 1992; Rebers *et al*. 1994, 1995). Among them, GA_4 seemed to be the major GA, and GA_1, GA_9 and GA_{34} 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 GA_4 and its inactivation product GA_{34} were observed only in growing floral stems of cooled tulip bulbs ('Apeldoorn'), and the absence of a significant quantitative accumulation of GA_1. Based on these results, they suggested that GA_4 is probably involved in floral stem elongation in tulip. Saniewski *et al*. (1990) found that GA_3 and GA_{4+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'). GAs 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 GAs 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. Endogenous levels of GAs induced during IAA action are efficient for control of stem growth in the interaction with the auxin, and additional exogenous level of GAs only slightly affected the stem growth induced by IAA. Ross *et al*. (2003) claimed that in elongating internodes of pea (*Pisum sativum* L.), auxin maintains the level of the bioactive GA, GA_1, by promoting GA_1 biosynthesis and by inhibiting GA_1 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 (GA_3) 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 Kamisaka 1988a, 1988b, 1990; Miyamoto *et al*. 1992). The role of GAs in the IAA-induced growth of stem segments prepared from cooled (matured) and uncooled bulbs, 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.

ACKNOWLEDGEMENTS

The research was partially supported by Research Institute of Pomology and Floriculture, Skierniewice, Poland (Project 23).

REFERENCES

Aloni B, Daie J, Wyse RE (1986) Enhancement of ^{14}C-sucrose export from source leaves of *Vicia faba* by gibberellic acid. *Plant Physiology* **82**, 962-966

Aung LH, de Hertogh AA (1979) Temperature regulation of growth and endogenous abscisic acid-like content of *Tulipa gesneriana* L. *Plant Physiology* **63**, 1111-1116

Banasik L, Saniewski M (1985) The effect of different auxins on tulip stalk elongation. *Acta Horticulturae* **167**, 193-204

Cocozza Talia M, Stellacci P (1979) A research on the effect of gibberellin upon tulip flowering. *Acta Horticulturae* **91**, 167-172

Geng XM, Okubo H, Saniewski M (2005) Cultivar and seasonal differences in the response on non pre-cooled tulip bulbs to gibberellin, TIBA and root excision. *Journal of the Faculty of Agriculture, Kyushu University* **50**, 503-509

Geng XM, Sato A, Okubo H, Saniewski M (2007) Changes in carbohydrate and ABA content in tulip bulbs during storage. *Journal of the Faculty of Agriculture, Kyushu University*, in press

Gilford J McD, Rees AR (1973) Growth of tulip shoot. *Scientia Horticulturae* **1**, 143-156

Hanks GR, Rees AR (1977) Stem elongation in tulip and narcissus: the influence of floral organs and growth regulators. *New Phytologist* **78**, 579-591

Jones SK, Hanks GR (1984) Treatment of tulips with gibberellic acid by vacuum infiltration. *Journal of Horticultural Science* **59**, 241-252

Kamerbeek GA, Beijersbergen JCM, Schenk PK (1972) Dormancy in bulbs and corms. In: Goren N, Mehdel K (Eds) *Proceeding of XVIIIth Intl. Horticultural Congress*, Tel Aviv, Vol V, pp 233-239

Miyamoto K, Kamisaka S (1988a) Stimulation of *Pisum sativum* epicotyl elongation by gibberellin and auxin. – Different effects of two hormones on osmoregulation and cell walls. *Physiologia Plantarum* **74**, 457-466

Miyamoto K, Kamisaka S (1988b) Growth and osmoregulation in *Pisum sativum* subhooks as affected by gibberellic acid and cotyledon excision. *Physiologia Plantarum* **74**, 669-674

Miyamoto K, Kamisaka S (1990) Effect of gibberellic acid on epicotyl growth and carbohydrate distribution in derooted *Pisum sativum* cuttings with or without cotyledons. *Physiologia Plantarum* **80**, 357-364

Miyamoto K, Ueda J, Kamisaka S (1992) Sugar accumulation in growing subhooks of etiolated *Pisum sativum* seedlings – Stimulation of sugar exudation and invertase activity in epicotyls by gibberellic acid. *Physiologia Plantarum* **84**, 106-112

Miyamoto K, Oka M, Ueda J (1997) Update of the possible mode of action of the jasmonates: Focus on the metabolism of cell wall polysaccharides in relation to growth and development. *Physiologia Plantarum* **100**, 631-638

Moore TC (Ed) (1989) Abscisic acid and related compounds. In: *Biochemistry and Physiology of Plant Hormones* (2nd Edn), Springer-Verlag, New York, pp 196-227

Mulligan DR, Patrick JW (1979) Gibberellic-acid-promoted transport of assimilates in stems of *Phaseolus vulgaris* L. Localized versus remote site(s) of action. *Planta* **145**, 233-238

Ohyama T, Komiyama S, Ohtake N, Sueyoshi K, Teixeira da Silva JA, Ruamrungsri S (2006) Physiology and genetics of carbon and nitrogen metabolism in tulip. In: Teixeira da Silva JA (Ed) *Floriculture, Ornamental and Plant Biotechnology Advances and Topical Issues* (1st Edn, Vol III), Global Science Books, Isleworth, UK, pp 12-25

Okubo H, Shiraishi S, Uemoto S (1986) Factors controlling elongation of the last internode in tulip flower stalk. *Journal of the Japanese Society of Horticultural Science* **55**, 320-325

Okubo H, Uemoto S (1984) Effects of darkness on stem elongation in tulip. *Scientia Horticulturae* **23**, 391-397

Okubo H, Uemoto S (1985) Changes in endogenous gibberellin and auxin activities during first internode elongation in tulip flower stalk. *Plant Cell Physiology* **25**, 709-719

Op den Kelder P, Benschop M, de Hertogh AA (1971) Factors affecting floral stalk elongation of flowering tulips. *Journal of the American Society of Horticultural Science* **96**, 603-605

Rebers M (1992) The role of gibberellins in the cold requirement of tulip. *Acta Horticulturae* **325**, 253-258

Rebers M, Vermeer E, Knegt E, Shelton CJ, van der Plas LHW (1994) Gibberellins in tulip bulb sprouts during storage. *Phytochemistry* **36**, 269-272

Rebers M, Vermeer E, Knegt E, Shelton CJ, van der Plas LHW (1995) Gibberellins level and cold-induced floral stalk elongation in tulip. *Physiologia Plantarum* **94**, 687-691

Rietveld PL, Wilkinson C, Franssen HM, Balk PA, van der Plas LHW, Weisbeek PJ, de Boer AD (2000) Low temperature sensing in tulip (*Tulipa gesneriana* L.) is mediated through an increased response to auxin. *Journal of Experimental Botany* **51**, 587-594

Ross JJ, O'Neill DP, Rathbone DA (2003) Auxin-gibberellin interactions in pea: integrating the old with the new. *Journal of Plant Growth Regulation* **22**, 99-108

Rudnicki RM, Nowak J, Saniewski M (1976) The effect of gibberellic acid on sprouting and flowering of some tulip cultivars. *Scientia Horticulturae* **4**, 387-397

Saniewski M (1989) The use of paclobutrazol, an inhibitor of gibberellin biosynthesis, for study of hormonal control of tulip stem elongation. *Bulletin of the Polish Academy of Sciences, Biological Sciences* **36**, 265-269

Saniewski M, de Munk WJ (1981) Hormonal control of shoot elongation in tulips. *Scientia Horticulturae* **15**, 363-372

Saniewski M, Horbowicz M (2005) Changes in endogenous flavonoids level during cold storage of tulip bulbs. *Acta Horticulturae* **669**, 245-251

Saniewski M, Kawa-Miszczak L (1992) Hormonal control of growth and development of tulips. *Acta Horticulturae* **325**, 43-54

Saniewski M, Kawa L, Węgrzynowicz E (1990) Stimulatory effect of gibberellins on tulip stem elongation. *Prace Instytutu Sadownictwa i Kwiaciarstwa, Seria B – Rośliny Ozdobne* **15**, 104-112

Saniewski M, Kawa-Miszczak L, Węgrzynowicz-Lesiak E, Okubo H (1999) Inhibitory effect of abscisic acid on shoot growth and flowering induced by gibberellic acid in non-cooled derooted bulbs of tulip (*Tulipa gesneriana* L.). *Journal of the Faculty of Agriculture, Kyushu University* **44**, 25-32

Saniewski M, Okubo H (1997) Auxin induces stem elongation in nonprecooled and precooled derooted and rooted tulip bulbs. *Journal of the Faculty of Agriculture, Kyushu University* **42**, 53-61

Saniewski M, Okubo H, Miyamoto K, Ueda J (2005a) Auxin induces growth of stem excised from growing shoot of cooled tulip bulbs. *Journal of the Faculty of Agriculture, Kyushu University* **50**, 481-488

Saniewski M, Ueda J, Miyamoto K, Okubo H, Puchalski J (2005b) Methyl jasmonate induced gum and inhibits stem growth promoted by auxin in uncooled tulip bulbs. *Journal of the Faculty of Agriculture, Kyushu University* **50**, 41-47

Saniewski M, Węgrzynowicz-Lesiak E (1993) Continuous supply of auxin is necessary for tulip stem growth. *Journal of Fruit and Ornamental Plant Research* **1**, 59-66

Skrzypek E, Miyamoto K, Saniewski M, Ueda J (2005) Identification of jasmonic acid and its methyl ester as gum-inducing factors in tulips. *Journal of Plant Research* **118**, 27-30

Terry PH, Aung LH, de Hertogh AA (1982) Identification of abscisic acid in *Tulipa gesneriana* L. by gas-liquid chromatography and mass spectroscopy. *Plant Physiology* **70**, 1574-1576

van Bragt, Van Ast KJ (1976) Substitution of the cold requirement of tulip cv. Apeldoorn by GA_3. *Scientia Horticulturae* **4**, 117-122

van Bragt J, Zijlstra FA (1971) Effects of gibberellins on flowering of tulip cv. Apeldoorn. *Zeitschrift für Pflanzenphysiology* **64**, 139-144