РАСТЕНИЕВЪДНИ НАУКИ, ГОД. LI, No. 6
PLANT SCIENCE, VOL. LI, No. 6
София. 2014. Sofia

EFFECT OF INHIBITORS OF AUXIN POLAR TRANSPORT ON THE GROWTH INDUCED BY INDOLE-3-ACETIC ACID AND INDOLE-3-BUTYRIC ACID IN EXCISED STEM OF TULIPS

MARIAN SANIEWSKI*[1], ELŻBIETA WĘGRZYNOWICZ-LESIAK*, JUSTYNA GÓRAJ*,
KENSUKE MIYAMOTO**, JUNICHI UEDA***
**Research Institute of Horticulture, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland*
***Faculty of Liberal Arts and Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan*
****Graduate School of Science, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan*
[1]E-mail: Marian.Saniewski@inhort.pl

Abstract

In the present study we report the effect of inhibitors of auxin polar transport, NPA, TIBA and morphactin IT 3456, on indole-3-acetic acid (IAA)- and indole-3-butyric acid (IBA)-induced growth of excised stem from growing tulips (*Tulipa gesneriana* L.) cv. Apeldoorn, after removal of flower bud and all leaves. Auxins, IAA and IBA, at a concentration of 0.1% in lanolin paste were applied on the top of explants and inhibitors of auxin polar transport in the middle of the 4th internode, and explants were kept in water. IAA and IBA similarly induced growth of tulip stem isolated from growing shoots and all inhibitors tested substantially inhibited the growth of lower internodes (the 1st, 2nd and 3rd) but stimulated growth of the 4th (last) internode. These data suggest that these inhibitors of auxin polar transport blocked auxin transport from the 4th internode and in consequence higher levels of auxin accumulated in the 4th internode stimulated the growth of the internode.

Key words: auxin polar transport inhibitors, indole-3-acetic acid, indole-3-butyric acid, methyl 2-chloro-9-hydroxyfluorene-9-carboxylate, N-1-naphthylphthalamic acid, stem growth, 2,3,5-triiodobenzoic acid

During the development of tulip flowers three phases can be distinguished: the initiation and formation of a new sprout with flower (at high temperature), the internal preparation for stem elongation (at low temperature), the rapid elongation of shoot (at high temperature). Tulip bulbs, with a terminal bud containing a complete flower require 12-16 weeks of low temperature for shoot elongation, this suggests that a kind of dormancy that can be released by exposure to low temperature (Figure 1) (De Hertogh, 1974). The leaves and gynoecium provide auxins which control the elongation of the stem in tulip (Op den Kelder et al., 1971; Hanks and Rees, 1977; Saniewski and De Munk, 1981; Banasik and Saniewski, 1985). Excision of the flower bud and all leaves in the early stage of tulip growth resulted in almost total inhibition of stem growth, and this inhibition was almost completely recovered by the exogenous application of auxin to the place where the flower bud was removed (Saniewski and De Munk, 1981; Banasik and Saniewski, 1985). It was found also that auxin induced the growth of stem segments excised from growing shoot of cooled tulip bulbs and in stem segments excised from cooled and uncooled tulip bulbs (Gabryszewska and Saniewski, 1983; Saniewski et al., 2005; 2007).

To clarify the role of auxin in tulip stem growth, intensive studies using auxin polar transport inhibitors, such as morphactin (methyl 2-chloro-9-hydroxyfluorene-9-carboxylate, morphactin IT 3456), 2,3,5-triiodobenzoic acid (TIBA) and *N*-1-naphthylphthalamic acid (NPA), have been performed. TIBA applied at the node between the first and the second internodes of tulip flower stalk significantly inhibited the dark-induced elongation and reduced the levels of diffusible auxin from the upper internodes to the first internode (Okubo and Uemoto, 1985). It was also shown that TIBA, NPA and morphactin strongly inhibited the growth of internodes induced by indole-3-acetic acid (IAA) in cooled rooted tulip bulbs after removal of flower bud and all leaves (Saniewski and Okubo, 1997; 1998a; 1998b; Saniewski et al., 1999). The inhibitory effect of TIBA, NPA and morphactin was restored by additional application of IAA treated below the place of the application of auxin transport inhibitors. Recently, it was found that TIBA, NPA and morphactin evidently stimulated the growth of the excised 4th internode (after removal of flower bud) when kept in water in normal and inverted position (Węgrzynowicz-Lesiak et al., 2013).

Indole-3-acetic acid (IBA) is the most widely used synthetic auxin for rooting purpose in agriculture (Lud-

wig-Müller and Epstein, 1991). IBA has also been identified as an endogenous compound acting as auxin in various plants. In the present study we report the effect of auxin polar transport inhibitors, TIBA, NPA and morphactin IT 3456, on IAA- and IBA-induced growth of stem excised from growing tulips, after removal of flower bud and all leaves.

MATERIAL AND METHODS

Bulbs of tulip (*Tulipa gesneriana* L.) cv. Apeldoorn with a circumference of 10 – 11 cm, after lifting, were stored at 18 – 20 °C for flower bud formation and other organs, and on October 19 transferred to 5 °C for dry cooling. After full cooling, starting from January 9, dry scales were removed and the bulbs were individually planted in pots and cultivated at 18 – 20 °C in a greenhouse under natural light conditions. When the length of shoots was about 7.0 – 8.0 cm (measured from base of basal plate) shoots were excised at the basal plate from the growing tulips, and then all leaves and flower bud were removed. In the place of removed flower bud, a small amount of lanolin (control) or lanolin containing indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) at a concentration of 0.1% in lanolin was applied. In the excised stem treated with IAA or IBA in the middle of the 4th internode were applied inhibitors of auxin polar transport, *N*-1-naphthylphthalamic acid (NPA), 2,3,5-triiodobenzoic acid (TIBA) and methyl 2-chloro-9-hydroxyfluorene-9-carboxylate (morphactin IT 3456) at a concentration of 0.2% in lanolin paste as a 1mm ring. After the treatments, the basal part of excised stems was kept in distilled water until the end of the experiments. Seven excised stems were used in each treatment and the experiments were repeated twice. Measurements of length of all four internodes were made during two weeks (to the end of stem growth), and the length of internodes in different treatments was subjected to an analysis of variance. Duncan's multiple range test was used for the mean separation at $p = 0.05$.

RESULTS AND DISCUSSION

Indole-3-butyric acid (IBA) has been confirmed as an endogenous auxin in a variety of plant species, but until now there is lack of data about its occurrence in tulip (Ludwig-Müller, 2000). Banasik and Saniewski (1985) showed that similarly to IAA, IBA induced stem growth of tulip when it was applied to the top of stem after excision of flower bud and all leaves in growing tulips. Recently, Saniewski et al. (2012) documented that IBA similarly to IAA induced tulip stem growth isolated from growing shoots, and the growth was substantially inhibited by inhibitors of gibberellin (GA) biosynthesis, paclobutrazol, flurprimidol and prohexadione-Ca.

As shown in Figures 2 and 3, exogenously applied IAA and IBA substantially induced elongation of all internodes. The growth of the 1st to the 3rd internodes induced by simultaneous application of IAA and IBA with auxin polar transport inhibitors observed on Feb. 01 (on 8th day after the application) was significantly inhibited compared with that by IAA and IBA alone. On the contrary, the growth of the 4th internode induced by simultaneous application of IAA or IBA with auxin polar transport inhibitors observed on Feb. 01 was almost same as that by IAA and IBA alone.

The growth of the 1st to the 3rd internodes induced by simultaneous application of IAA and IBA with auxin polar transport inhibitors observed on Feb. 05 (on 12th day after the appliation) was almost same as that on Feb. 01. At this time, however, the growth of the 4th internode induced by simultaneous application of IAA and IBA with auxin polar transport inhibitors was significantly promoted compared to that on Feb. 01. In addition, the growth of the 4th internode induced by simultaneous application of IAA and IBA with auxin polar transport inhibitors was extremely promoted compared to that by IAA and IBA alone. The mechanisms of extreme enhancement of the growth in the 4th internode induced by simultaneous application IAA and IBA with auxin polar transport inhibitors observed on Feb. 05 but not on Feb. 01 have not been clear yet but a possible explanation will be proposed.

IBA has been identified as an endogenous compound in various plants (Ludwig-Müller and Epstein, 1991, Ludwig-Müller, 2000). Yang and Davies (1999) showed that aqueous IBA, directly applied to the growing internodes of pea via a cotton wick, to be nearly as effective as IAA in inducing of stem elongation. IBA showed promotive effect to the same extent to IAA at same concentration (0.1%) in tulip stem segments (Figs. 2 and 3). This suggests that IBA acts as auxin by a similar manner to IAA, whereas it has been reported that the conversion of IBA to IAA occurs in many plants (Ludwig-Müller, 2000). Further studies for explanation either IBA itself is active or that it modulates the activity of IAA as well as studies on its occurrence in tulips are required.

The growth of the 1st to the 3rd internodes induced by IAA and IBA was substantially inhibited by the application of auxin polar transport inhibitors in the middle of the 4th internode, but the growth of the 4th internode was extremely promoted. This result suggests that auxin polar transport inhibitors affect auxin levels in the 1st to the 4th internodes by blocking auxin transport from the 4th internode, resulting in the higher accumulation of auxin in the 4th internode. In consequence the higher level of auxin accumulated in the 4th internode which may stimulate directly or indirectly (through gibberellins) the elongation of the internode. The mechanism by which TIBA, NPA and morphactin inhibit auxin transport is not fully known. NPA binds specifically to so-called NPA receptor, thereby inhibiting the carrier-mediated efflux of IAA; IAA dose not compete with NPA for binding site and NPA-binding site is important

for auxin transport (Muday et al., 1993; Lomax et al., 1995; Ruegger et al., 1997). Morphactin has also been shown to bind to NPA receptor, suggesting that morphactin inhibits auxin polar transport as NPA (Thomson and Leopold, 1974; Sussman and Goldsmith, 1981). On the other hand, it has been suggested that TIBA and NPA have different binding site (Thomson et al., 1973; Michalke et al., 1992). Further studies relating to the determination of endogenous levels of auxin after application of inhibitors of auxin polar transport as well as the mode of action of these inhibitors to block auxin polar transport will be required.

Based on the results in this study, it is strongly suggested that significant promotion in the growth of the 4th internode induced by simultaneous application of IAA and IBA with auxin polar transport inhibitors observed on Feb. 05 results in the consequence of the interaction of the growth between the 1st ~ the 3rd internodes and

On left, and fully dry cooled bulbs (stored at 5 °C) – on right;
bulbs planted on January 20, photographed on February 15

Fig. 1. Tulip growth after planting in greenhouse of uncooled bulbs (bulbs stored at about 18 °C)

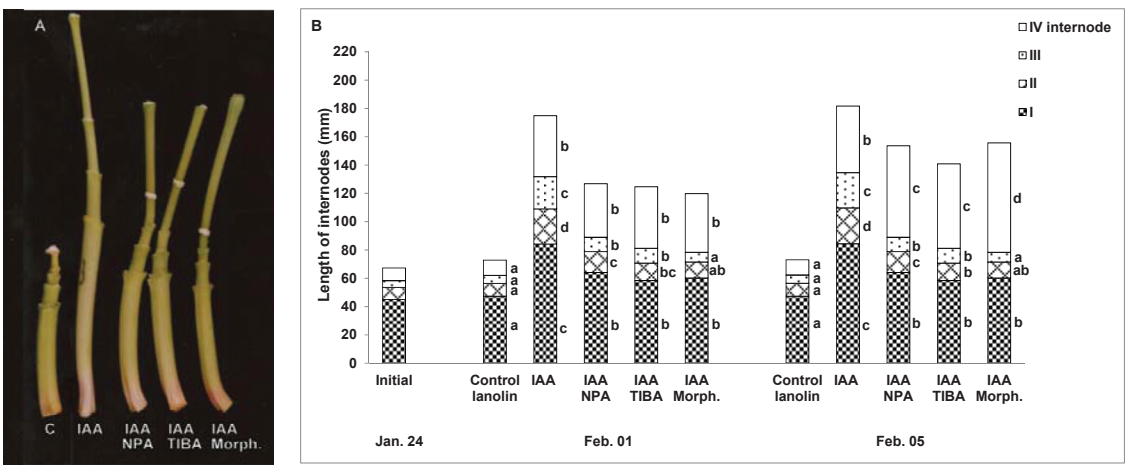

Fig. 2. The effect of NPA, TIBA and morphactin IT 3456 at a concentration of 0.2% applied in the middle of 4th internode, on the growth of tulip stem excised from growing plants when the growth was induced by IAA (0.1%) applied in the place of removed flower bud and after removal of all leaves; planting – Jan. 9, treatments – Jan. 24
A – picture made on February 4; B – diagram of the growth of internodes in different treatments.

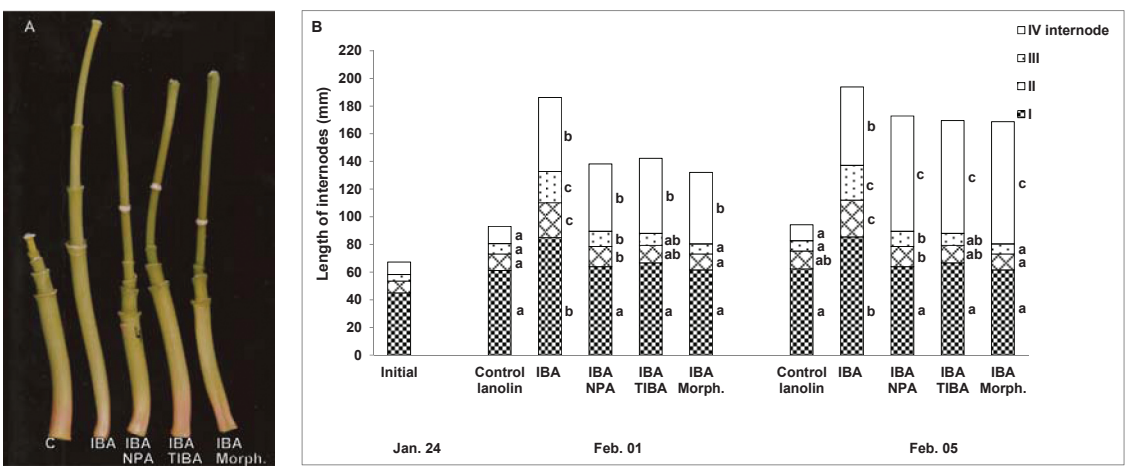

Fig. 3. The effect of NPA, TIBA and morphactin IT 3456 at a concentration of 0.2% applied in the middle of 4th internode, on the growth of tulip stem excised from growing plants when the growth was induced by IBA (0.1%) applied in the place of removed flower bud and after removal of all leaves; planting – Jan. 9, treatments – Jan. 24.
A – picture made on February 4; B – diagram of the growth of internodes in different treatments.

the 4th internode. Nutrition, carbon source and plant hormones supplied from non-growing tissues have been suggested to affect the stem growth induced by auxin. On the basis of dry weight of different internodes of untreated and IAA treated stems, carbohydrates occurring in the 1st internode have been clarified to utilize for the growth of the upper internodes especially the 3rd and the 4th internodes with auxin (Saniewski et al., 2005). Endogenous levels of gibberellins (GAs) have been shown to be responsible for the translocation of solutes in plants. Considerable levels of endogenous active GAs have also been suggested to contribute to tulip stem growth with endogenous auxins (Saniewski, 1989; Saniewski et al., 2010).

Close relationships between auxin polar transport inhibitors and endogenous GAs have been intensively studied. Auxin polar transport inhibitors have been suggested to inhibit (affect) endogenous GA levels and/or GA biosynthesis. Ross (1998) showed that auxin transport inhibitors, NPA, TIBA and 9-hydroxyfluorene-9-carboxylic acid (HFCA) when applied to elongation internodes of intact wild type of pea plants, markedly reduced internode elongation and endogenous level of auxin and bioactive gibberellin A_1 (GA_1), below the application sites. Author suggests that the inhibitors of auxin transport may affect GA_1 transport from the apical bud or GA_{20} metabolism in stem. TIBA substantially decreased the asymmetric distribution of IAA and the gradient of *OsGA3ox1* expression in rice leaf sheath, suggesting that the asymmetric distribution of auxin affected by gravistimulation induced a gradient of GAs via asymmetric expression of *OsGA3ox1* (Dayong et al., 2005). The effects of auxin transport inhibitors on GA20-oxidation were also examined in wild-type and *tir3-1* seedlings of *Arabidopsis*. NPA and TIBA lead to overexpression of the GA-biosynthetic gene *AtGA20ox1* comparable in magnitude to the overexpression observed in seedlings treated with a GA biosynthesis inhibitor such as paclobutrazol, suggesting that at least in some tissues auxin polar transport inhibitors, directly or indirectly, may reduce the level of bioactive GA and/or alter GA signal transduction (Isabel et al., 2005). The application of NPA to unpollinated ovaries induced parthenocarpic fruit-set of tomato, associated with an increase in IAA content, and that this effect was negated by paclobutrazol. NPA-induced ovaries contained higher content of GA_1 and transcripts of GA biosynthetic genes (*SlCPS*, *SlGA20ox1*, and -*2*) (Juan et al., 2010).

CONCLUSIONS

Judging from the evidence described above together with the results in this study, the mode of action of auxin polar transport inhibitors to stimulate the growth of the 4th internode induced by exogenously applied auxin might be related to their regulations of endogenous levels of gibberellin and/or gibberellin biosynthesis. Further studies focusing on GA will be required.

REFERENCES

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

Dayong, C., Steven, J. N., Zhangcheng, T. and Weiming, C. 2005. Gibberellin-regulated XET is differentially induced by auxin in rice leaf sheath bases during gravitropic bending. *Journal of Experimental Botany*, 56, 1327-1334

De Hertogh, A. 1974. Principles for forcing tulips, hyacinths, daffodils, Easter lilies and Duch irises. *Scientia Horticulturae*, 2, 313-355

Gabryszewska, E. and Saniewski, M. 1983. Auxin control of tulip stalk elongation *in vitro*. *Scientia Horticulturae*, 19, 153-159

Hanks, G. R. and Rees, A. R. 1977. Stem elongation in tulip and narcissus: the influence of floral organs and growth regulators. *New Phytologist*, 78, 579-591

Isabel, D.-P., Suntara, E., Jeremy, P. C., Andrew, L. P., Peter, H. and Valerie, M. S. 2005. The auxin *transport inhibitor response 3* (*tir3*) allele of *BIG* and auxin transport inhibitors affect the gibberellin status of *Arabidopsis*. *Plant Journal*, 41, 231-242

Juan, C. S., Esther, C., Omar, R.-R., Lina, G.-G., La´zaro, E. P. P., Jose´ Luis, G.-M. 2010. Inhibition of auxin transport from the ovary or from the apical shoot induces parthenocarpic fruit-set in tomato mediated by gibberellins. *Plant Physiology*, 153, 851-862

Lomax, T. L., Muday, G. K. and Rubery, P. H. 1995. Auxin transport. In P. J. Davies (ed.). "Plant Hormones". *Kluwer Academic Publishers*, Dordrecht, The Netherlands, pp. 509-530

Ludwig-Müller, J. 2000. Indole-3-butyric acid in plant growth and development. *Plant Growth Regulation*, 32, 219-230

Ludwig-Müller, J. and Epstein, E. 1991. Occurrence and in vivo biosynthesis of indole-3-butyric acid in corn (*Zea mays* L.), *Plant Physiology*, 97, 765-770

Michalke, W., Katekar, G. F. and Geissler, A. E. 1992. Phytotropin-binding sites and auxin transport in *Cucurbita pepo*: evidence for two recognition sites. *Planta*, 187, 254-260

Muday, G. K., Brunn, S. A., Haworth, P. and Subramanian, M. 1993. Evidence for a single naphthylphthalamic acid binding site on the zucchini plasma membrane. *Plant Physiology*, 103, 449-456

Okubo, H. and Uemoto, S. 1985. Changes in endogenous gibberellins and auxin activities during first internode elongation in tulip flower stalk. *Plant Cell Physiology*, 26, 709-719

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

Ross, J. J. 1998. Effects of auxin transport inhibitors on gibberellins in pea. *Journal of Plant Growth Regulation*, 17, 141-146

Ruegger, M., Dewey, E., Hobbie, L., Brown, D., Bernasconi, P., Turner, J., Muday, G. and Estelle, M. 1997. Reduced naphthylphthalamic acid binding in the *tir3* mutant of *Arabidopsis* is associated with a reduction in polar transport and diverse morphological defects. *Plant Cell*, 9, 745-757

Saniewski, M. 1989. The use of paclobutrazol, an inhibitor of gibberellins biosynthesis, for study of hormonal control of tulip stem elongation. *Bull. Pol. Acad. Sci. Biol.,* 37, 55-64

Saniewski, M. and de Munk, W. J. 1981. Hormonal control of shoot elongation in tulips. *Scientia Horticulturae*, 5, 363-372

Saniewski, M., Góraj, J., Węgrzynowicz-Lesiak, E., Okubo, H., Miyamoto, K. and Ueda, J. 2010. Different growth of excised and intact fourth internode after removal of the flower bud in growing tulips: Focus on auxin action. *Journal of Fruit and Ornamental Plant Research*, 18, 297-308

Saniewski, M. and Okubo, H. 1977. Auxin induces stem elongation in nonprecooled and precooled derooted and rooted tulip bulbs. *J. Fac. Agr., Kyushu Univ.*, 42, 53-61

Saniewski, M. and Okubo, H. 1998a. Effects of 2,3,5-triiodobenzoic acid (TIBA) on stem growth induced by indole-3-acetic acid (IAA) and naphthylacetic acid (NAA) in precooled rooted tulip bulbs. *J. Fac. Agr., Kyushu Univ.*, 43, 11-23

Saniewski, M. and Okubo, H. 1998b. Inhibitory effect of naphthylphthalamic acid (NPA) on stem growth induced by auxin in precooled tulip bulbs. *J. Fac. Agr., Kyushu Univ.*, 43, 59-66

Saniewski, M., Okubo, H., Miyamoto, K. and Ueda, J. 2012. The role of endogenous gibberellin in tulip stem growth induced by IAA and IBA: relevance to inhibitors of gibberellin biosynthesis. *Acta Horticulturae*, 932, 397-403

Saniewski, M., Okubo, H., Miyamoto, K. and Ueda J. 2005. Auxin induced growth of stem excised from growing shoot of cooled tulip bulbs. *J. Fac. Agr., Kyushu Univ.*, 50, 481-488

Saniewski, M., Okubo, H., Miyamoto, K. and Ueda, J. 2007. Susceptibility and/or responsiveness of tulip stem segments excised from cooled and uncooled bulbs to indole-3-acetic acid. *Floric. Ornam. Biotechnol.,* 1, 142-146

Saniewski, M., Okubo, H. and Puchalski, J. 1999. Effect of morphactin on stem growth in relation to auxin in precooled rooted tulip bulbs. *Acta Physiologiae Plantarum*, 21, 167-174

Sussman, M. R. and Goldsmith, M. H. M. 1981. Auxin uptake and action of N-1-naphthylphthalamic acid in corn coleoptiles. *Planta*, 150, 15-25

Thomson, K.-S, Hertel, R., Muller, S. and Tavares, J. E. 1973. N-naphthylphthalamic acid and 2,3,5-triiodobenzoic acid. *In vitro* binding to particulate cell fractions and action on auxin transport in corn coleoptiles. *Planta*, 109, 337-352

Thomson, K.-S. and Leopold, A. C. 1974. *In vitro* binding of morphactins and 1-N-naphthylphthalamic acid in corn coleoptiles and their effects on auxin transport. *Planta*, 115, 259-270

Węgrzynowicz-Lesiak, E., Góraj, J., Miyamoto, K., Ueda, J. and Saniewski, M. 2013. Effects of auxin polar transport inhibitors on the growth of the excised fourth internode in tulips. *Journal of Horticultural Research*, 21, 31-39

Yang, T. and Davies, P. J. 1999. Promotion of stem elongation by indole-3-butyric acid in intact plants of *Pisum sativum*. *Plant Growth Regulation*, 27, 157-160