EFFECTS OF SUGARS ON THE GROWTH AND CHLOROPHYLL CONTENT IN EXCISED TULIP STEM IN THE PRESENCE OF INDOLE-3-ACETIC ACID

Elżbieta Węgrzynowicz-Lesiak[1], Marian Saniewski[1], Justyna Góraj[1], Marcin Horbowicz[2], Kensuke Miyamoto[3] and Junichi Ueda[4]

[1]Research Institute of Horticulture
Konstytucji 3 Maja 1/3, 96-100 Skierniewice, POLAND
[2]Department of Plant Physiology and Genetics, Institute of Biology, Siedlce University of Natural Sciences and Humanities, Prusa 12, 08-110 Siedlce, POLAND
[3]Faculty of Liberal Arts and Sciences, Osaka Prefecture University,
1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, JAPAN
[4]Graduate School of Science, Osaka Prefecture University,
1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, JAPAN

(Received May 16, 2012/Accepted June 15, 2012)

A B S T R A C T

The purpose of this study was to clarify the effect of sucrose on auxin-induced growth of stem excised from growing tulips and excised directly from cooled and not cooled bulbs, and on the growth of excised IV internode from growing plants in the presence of auxin. In all cases flower bud was replaced by IAA (indole-3- acetic acid, 0.1%, w/w in lanolin) and basal part of excised segments of stem was kept in distilled water or in solution of various sugars at different concentrations. IAA-induced growth of excised stems isolated from growing tulips was inhibited by sucrose at concentrations of 5.0% and 10.0%, but sucrose at 1.25% and 2.5% did not. Sucrose at all concentrations used evidently delayed senescence and increased chlorophyll contents in excised stems in the presence of IAA. Sucrose induced stiffing in isolated stems in the presence of IAA, and much less infective by pathogen in comparison to stem treated with IAA only. Mannitol and sorbitol at concentrations of 5.0% and 10.0% substantially inhibited IAA-induced growth of stem segments. Stem segments excised from cooled and not cooled tulip bulbs were more sensitive than those isolated from growing shoots due to application of sucrose and glucose; more inhibitory effect was observed. Sucrose at concentrations of 5.0% and 10.0% only slightly inhibited growth of IV internode treated with IAA and all concentrations of sucrose (1.25%, 2.5%, 5.0% and 10.0%) substantially increased chlorophyll content. The possible mode of actions of sucrose interacting with auxin to regulate stem growth is also discussed although sugar response is complicated by the fact that plants have multiple sugar-response pathways.

Key words: indole-3-acetic acid (IAA), stem explants, tulip, growth, sucrose, glucose, chlorophyll

INTRODUCTION

In tulip bulbs with terminal buds containing a complete flower, cold treatment for a period of 12-16 weeks is required for floral stalk elongation (De Hertogh, 1974). Enlargement of the stem and leaves of cooled tulip bulbs is entirely due to the elongation of cells produced in earlier developmental stages (Gilford and Rees, 1973). 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 had been removed (Saniewski and De Munk, 1981; Banasik and Saniewski, 1985). The application of indole-3-acetic acid (IAA) to the cut surface of the top internode in stem segments prepared from growing shoots of cooled tulip bulbs substantially promoted the growth of all internodes (Saniewski et al., 2005), suggesting that auxins produced in flower buds and leaves, mostly gynoecium, are transported basipetally and regulate cell elongation in all internodes.

On the other hand, Saniewski et al. (2007) have recently reported that in stem segments excised from cooled and not cooled tulip bulbs stored for three months at 5 °C and 17 °C, respectively, after flower bud formation, the growth was enhanced by IAA applied to the place where buds were removed. They also reported that the elongation of excised IV internode with node or without node, after removal of the flower bud, was much higher than that of intact IV internode in growing tulip shoot without flower bud (Saniewski et al., 2010). Furthermore, IAA applied to the cut surface of excised IV internode, just after removal of flower bud, had little effect, or only slightly, on growth of the IV internode. The reason why there are some differences in the growth of excised and intact IV internode of tulip shoots after removal of the flower bud has not been clear yet.

On the basis of dry weight of different internodes of untreated and IAA-treated stems it is suggested that carbohydrates occurring in the first (basal) internode are utilized for the growth of the upper internodes, especially III and IV internodes induced by auxins. Soluble sugars, especially sucrose, play an important role in plant growth and development as a carrier of energy, carbon partitioning, and regulator of osmotic potential and signalling molecules. It is possible that the growth of excised tulip stem induced by IAA is limited by endogenous sugars that can be readily utilized in elongating cells as well.

In this study, we report the effect of sugars on the IAA-induced growth of stem excised from growing tulips and excised directly from cooled and not cooled bulbs, and on the growth of excised IV internode from growing

plants in the presence of auxin. Possible role of sugars in stem elongation in the presence of auxin is also discussed.

MATERIAL AND METHODS

Tulip bulbs (*Tulipa gesneriana* L. cv. Apeldoorn) with circumference of 10-11 cm after lifting, were stored at 18-20 °C until October 20 and then the following experiments (A-C) were carried out. After appropriate incubation, the length of all internodes was measured. In experiments (A-C), 10 to 12 segments were used and the respective experiment was repeated three times. In some experiments, the contents of chlorophylls and free phenolic compounds were determined.

The content of chlorophylls extracted from the middle part of III and IV internodes with acetone was determined spectrophotometrically (Bruinsma, 1963). Chlorophyll content was calculated for 1 cm internode explants. Five plants were used for determination of chlorophyll in each treatment.

The length of internodes and chlorophylls content were subjected to an analysis of variance and Duncan's multiple range test was used for means separation at $p = 0.05$.

Total amounts of phenolics in lyophilized stem tissues prepared from the middle part of III and IV internodes were determined by Folin–Ciocalteau method published by Singleton and Rossi (1965) and modified by Kaur and Kapoor (2002). Absorbance at 700 nm was measured and the results were expressed as contents of chlorogenic acid instead of catechol.

Experiment A

Tulip bulbs were transferred to 5 °C for dry cooling until planting. After full cooling of bulbs the tunics were removed and the bulbs were individually planted in pots and cultivated at 18-20 °C in a greenhouse under natural light conditions. 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 IAA (0.1%, w/w in lanolin) was applied. After the treatment, the excised stems were kept in distilled water and in different concentrations of sucrose, mannitol or sorbitol until the end of the experiment.

Experiment B

Tulip bulbs were transferred to 5 °C for dry cooling or stored at 17 °C (not cooled bulbs). After full cooling of bulbs and in the same time from not cooled bulbs, stem segments consisting of all internodes were excised, and flower buds were removed. Lanolin paste alone or containing IAA (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 kept in distilled water or in solutions containing different concentrations of sucrose and glucose until the end of the experiment.

Experiment C

Tulip bulbs were planted in field conditions. At different stages of growth the IV internode with or without node was excised from tulip shoots, and flower bud was removed. Lanolin

alone or containing IAA (0.1%, w/w) was applied to the cut surface of the IV internode instead of the flower bud. The basal part of the excised IV internode was kept in water or in sugar solutions as in Experiment B.

RESULTS AND DISCUSSION

Effect of soluble sugars on growth of tulip stems

As described previously (Saniewski et al., 2005), in isolated stem segments from growing tulips, kept in distilled water and treated with lanolin only (control), the growth of stem segments was very small. On the other hand, the application of IAA as a lanolin paste at the cut surface of flower bud greatly promoted the elongation (Fig. 1, Tab. 1 and 2).

Sucrose applied alone did not affect growth of isolated stem segments in comparison to that of segments kept in distilled water (data not shown). On the other hand, if the isolated stems treated with IAA were kept in solution of different concentrations of sucrose, differential reaction in stem growth depending on sucrose concentration was found. Sucrose at concentration of 1.25% and 2.5% did not affect growth of isolated stems. Sucrose at concentrations of 5% and 10%, however, evidently inhibited stem growth promoted by IAA, proportionally to sucrose concentration (Tab. 1 and 2, Fig. 1). Sucrose at higher concentrations also caused stiffening of isolated stem segments and decreased infection by pathogens in comparison to treatment with IAA only (data not shown).

It is worthwhile to report that sucrose at 10.0% did not inhibit elongation of IV internode in the presence of IAA, but inhibited growth of lower internodes (I, II, III). Differently from the effect on internode growth at the concentration of 10%, sucrose at concentration of 5% stimulated the growth of IV internode but inhibited the growth of all lower internodes in comparison to treatment with IAA alone; similar tendency being observed at 2.5% sucrose. These results suggest that interaction of sucrose and IAA on growth of isolated stem segments is completely different in each internode.

On the other hand, as well as sucrose, mannitol and sorbitol applied alone at concentrations of 5.0% and 10.0% had little effect on elongation growth of isolated stem segment in the absence of IAA (Tab. 3, Fig. 2). Similar to sucrose at 5 and 10%, both mannitol and sorbitol at concentrations of 5 and 10% substantially inhibited IAA-induced growth of isolated stem segments in a concentration-dependent manner; mannitol showing more inhibitory effect than sorbitol. It is possible that sucrose and glucose act by affecting osmotic potential in excised tulip segments induced by IAA, since mannitol and sorbitol, well known as osmotic regulators, mimicked the inhibitory effect of sucrose and glucose. Sorbitol, sucrose and glucose have already been found to inhibit hypocotyl elongation of *Arabidopsis* (Gibson, 2005). These facts suggest that inhibition of elongation of isolated tulip stems by high concentration of soluble sugars is partially due to osmotic stress and metabolic suppression.

Figure 1. Interaction of IAA and sucrose on the elongation of stems excised from growing tulips and the chlorophyll content. IAA was applied in the place of removed flower bud and the explants were kept in water (control) or sucrose at different concentrations (photographed on March 25):

a) Control (lanolin) − water
b) IAA 0.1% − water
c) IAA 0.1% − sucrose 10.0%
d) IAA (0.1%) − sucrose 5.0%
e) IAA 0.1% − sucrose 2.5%
f) IAA 0.1% − sucrose 1.25%

Table 1. Interaction of IAA and sucrose (S) on the elongation of stems excised from growing tulips and the chlorophyll content. IAA was applied in the place of removed flower bud and the explants were kept in water (control) or sucrose at different concentrations

Treatment	Initial length of total stem on Feb. 27th [mm]	Measurements on March 17th [mm]					The content of chlorophylls (a+b) [µg/cm length] of the III and IV internodes measured on March 17th	
		internodes				total stem length		
		I	II	III	IV		III	IV
Control (lanolin) − water	61.7 a	40.4 ab	8.9 a	6.9 a	12.0 a	68.4 a	–	–
IAA 0.1% − water	69.8 ab	64.9 d	23.8 d	23.4 d	36.9 b	149.1 d	5.09 a	7.46 a
IAA 0.1% − S 10.0%	64.5 a	37.1 a	10.3 a	12.9 b	34.1 b	94.2 b	8.09 b	16.25 c
IAA 0.1% − S 5.0%	65.0 a	41.1 ab	14.9 b	19.2 c	57.4 d	132.7 c	12.69 c	11.48 b
IAA 0.1% − S 2.5%	74.0 b	48.4 c	20.1 c	24.8 d	58.8 d	152.1 d	8.50 b	8.15 a
IAA 0.1% − S 1.25%	70.0 ab	44.7 bc	20.0 c	28.3 e	50.0 c	143.0 cd	8.07 b	6.50 a

Table 2. Interaction of IAA and sucrose (S) on the elongation of stems excised from growing tulips and on the chlorophyll and free phenolic compounds content. IAA was applied in the place of removed flower bud and the explants were kept in water (control) or sucrose at different concentrations

Treatment	Initial length of total stem on March 28th [mm]	Measurements on April 11th [mm] internodes					The content of chlorophylls (a+b) [µg/cm length] of the III and IV internodes measured on				The content of free phenolic compounds in lyophilized samples of III and IV internodes on April 28th [mg/g dry weight]	
							April 15th		April 28th			
		I	II	III	IV	total stem length	III	IV	III	IV	III	IV
Control (lanolin) − water	89.6 b	57.0 a	14.4 a	14.7 a	21.4 a	107.5 a	4.19 a	2.89 a	–	–	–	–
IAA 0.1% − water	88.9 b	88.4 c	31.5 d	30.4 cd	62.1 b	212.4 d	6.07 a	6.50 b	2.85 a	6.32 a	14.92	16.85
IAA 0.1% − S 10.0%	83.5 ab	52.5 a	19.0 b	24.0 b	58.2 b	153.7 b	11.53 b	16.88 d	12.90 bc	26.88 d	–	–
IAA 0.1% − S 5.0%	83.5 ab	56.2 a	20.5 b	27.0 bc	78.0 cd	181.7 c	15.55 c	14.86 d	11.13 b	19.33 c	6.90	6.60
IAA 0.1% − S 2.5%	82.7 ab	56.4 a	26.0 c	33.9 d	88.4 d	204.6 d	12.57 b	10.55 c	13.95 c	10.55 b	6.90	6.05
IAA 0.1% − S 1.25%	79.7 a	66.9 b	31.9 d	34.2 d	70.1 bc	203.1 d	10.12 b	8.56 bc	11.82 b	10.62 b	11.0	11.90

Table 3. The effect of mannitol and sorbitol on the growth of tulip stem isolated from growing tulips and induced by IAA. IAA was applied in the place of removed flower bud and explants were kept in water (control) or solution of mannitol or sorbitol

Treatment	Initial length of total stem on February 16th [mm]	Total length of stem on February 25th [mm]	Increase of growth [mm]
Control (lanolin) − water	45.0 bc	53.1 c	8.1 a
Lanolin − mannitol 5.0%	40.8 ab	45.3 ab	4.5 a
Lanolin − mannitol 10.0%	45.0 bc	51.4 bc	6.4 a
Lanolin − sorbitol 5.0%	43.3 ab	52.8 c	9.5 a
Lanolin − sorbitol 10.0%	37.0 a	42.3 a	5.3 a
IAA 0.1% − water	50.5 c	129.4 g	78.9 e
IAA 0.1% − mannitol 5.0%	41.6 ab	78.9 e	37.3 c
IAA 0.1% − mannitol 10.0%	38.3 a	69.8 d	31.5 b
IAA 0.1% − sorbitol 5.0%	46.5 bc	111.4 f	64.9 d
IAA 0.1% − sorbitol 10.0%	42.5 ab	82.5 e	40.0 c

Figure 2. The effect of mannitol and sorbitol on the growth of tulip stem isolated from growing tulips and induced by IAA. IAA was applied in the place of removed flower bud and explants were kept in water (control) or solution of mannitol or sorbitol (photographed on February 25):

a) Control (lanolin) − water
b) Lanolin − mannitol 5.0%
c) Lanolin − mannitol 10.0%
d) Lanolin − sorbitol 5.0%
e) Lanolin − sorbitol 10.0%
f) IAA 0.1% − water
g) IAA 0.1% − mannitol 5.0%
h) IAA 0.1% − mannitol 10.0%
i) IAA 0.1% − sorbitol 5.0%
j) IAA 0.1% − sorbitol 10.0%

The IAA-induced growth of stem segments isolated from cooled and not cooled tulip bulbs was also greatly inhibited by sucrose (Tab. 4 and 5, Fig. 3 and 4). Glucose also inhibited IAA-induced growth of isolated stem segment from cooled and not cooled bulbs. Both sugars at concentrations of 5.0% and 10.0% totally inhibited tulip stem segments elongation induced by IAA. Thus, stem segments isolated directly from uncooled and cooled tulip bulbs are more sensitive to the application of sucrose after treatment with auxin than segments isolated from growing shoots

Table 4. Interaction of IAA and sucrose on the growth of the stem isolated from cooled and uncooled tulip bulbs. IAA was applied in the place of removed flower bud and the explants were kept in water (control) or sucrose at different concentrations

Treatment	Stem isolated from cooled bulbs on January 8th; initial length 14.4 mm Length of stem measured on Jan. 28th [mm]	Stem isolated from not cooled bulbs on January 8th; initial length 19.8 mm Length of stem measured on Jan. 28th [mm]
Control (lanolin) − water	23.3 a	27.7 a
Lanolin − sucrose 1.25%	24.4 a	27.4 a
Lanolin − sucrose 2.5%	18.4 a	23.0 a
Lanolin − sucrose 5.0%	15.6 a	22.7 a
Lanolin − sucrose 10.0%	16.0 a	21.1 a
IAA 0.1% - water	77.1 d	97.0 d
IAA 0.1% − sucrose 1.25%	67.4 c	70.1 c
IAA 0.1% − sucrose 2.5%	36.6 b	36.7 b
IAA 0.1% − sucrose 5.0 %	19.2 a	25.1 a
IAA 0.1% − sucrose 10.0%	15.6 a	21.4 a

Table 5. Interaction of IAA and glucose on the growth of the stem isolated from cooled and not cooled tulip bulbs. IAA was applied in the place of removed flower bud and the explants were kept in water (control) or glucose at different concentrations

Treatment	Stem isolated from cooled bulbs on January 9th; initial length 14.2 mm Length of stem measured on Jan. 28th [mm]	Stem isolated from not cooled bulbs on January 9th; initial length 21.3 mm Length of stem measured on Jan. 28th [mm]
Control (lanolin) − water	34.4 b	27.8 a
Lanolin − glucose 1.25%	20.1 a	25.7 a
Lanolin − glucose 2.5%	15.9 a	22.9 a
Lanolin − glucose 5.0%	15.9 a	22.2 a
Lanolin − glucose 10.0%	14.9 a	21.7 a
IAA 0.1% − water	73.4 c	111.3 d
IAA 0.1% − glucose 1.25%	69.9 c	82.9 c
IAA 0.1% − glucose 2.5%	41.9 b	40.0 b
IAA 0.1% − glucose 5.0 %	16.6 a	24.0 a
IAA 0.1% − glucose 10.0%	15.1 a	22.2 a

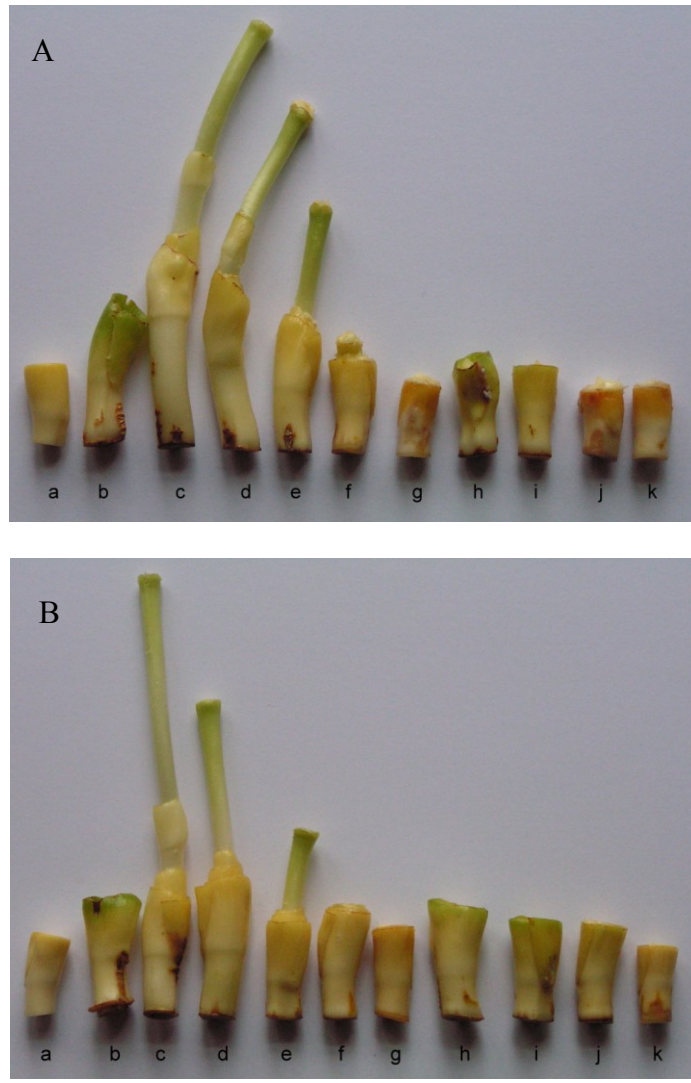

Figure 3. Interaction of IAA and sucrose on the growth of the stem isolated from cooled (A) and not cooled (B) tulip bulbs. IAA was applied in the place of removed flower bud and the explants were kept in water (control) or sucrose at different concentrations (photographed on January 27):

a) Initial segments
b) Control (lanolin) − water
c) IAA 0.1% − water
d) IAA 0.1% − sucrose 1.25%
e) IAA 0.1% − sucrose 2.5%
f) IAA 0.1% − sucrose 5.0%
g) IAA 0.1% − sucrose 10.0%
h) Lanolin − sucrose 1.25%
i) Lanolin − sucrose 2.5%
j) Lanolin − sucrose 5.0%
k) Lanolin − sucrose 10.0%

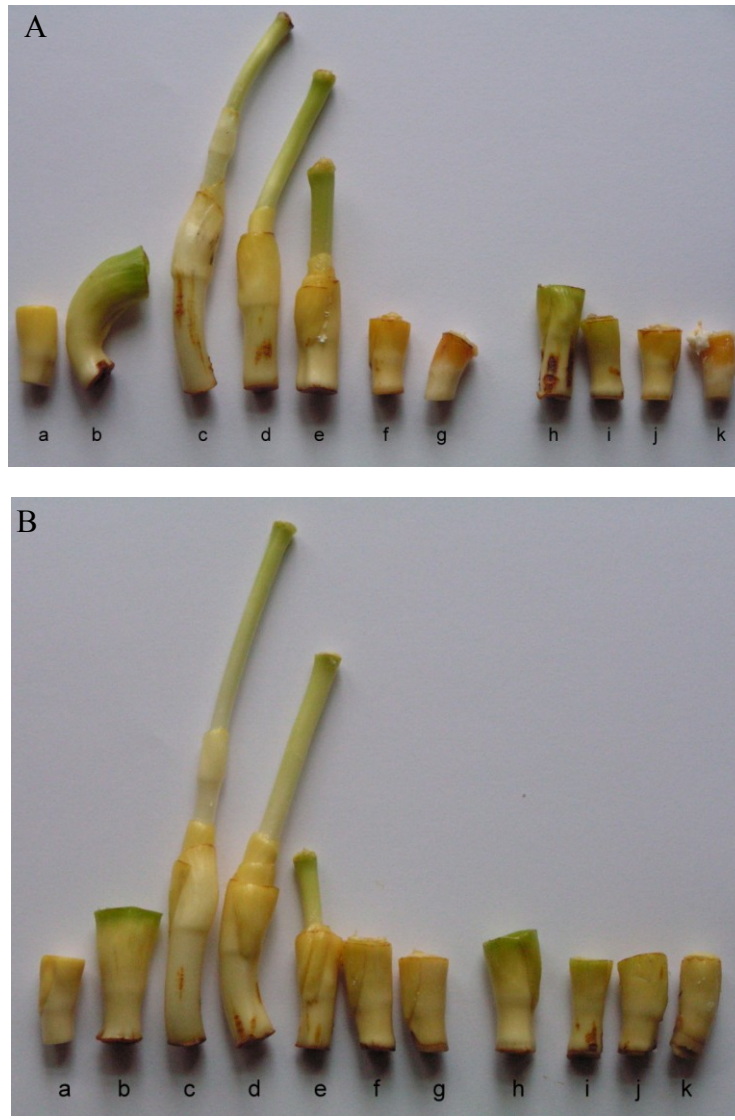

Figure 4. Interaction of IAA and glucose on the growth of the stem isolated from cooled (A) and not cooled (B) tulip bulbs. IAA was applied in the place of removed flower bud and the explants were kept in water (control) or glucose at different concentrations (photographed on January 27):

a) Initial segments
b) Control (lanolin) − water
c) IAA 0.1% − water
d) IAA 0.1% − glucose 1.25%
e) IAA 0.1% − glucose 2.5%
f) IAA 0.1% − glucose 5.0%
g) IAA 0.1% − glucose 10.0%
h) Lanolin − glucose 1.25%
i) Lanolin − glucose 2.5%
j) Lanolin − glucose 5.0%
k) Lanolin − glucose 10.0%

It has been documented previously that the higher growth of the excised IV internode, in comparison to that in growing tulips after removal of the flower bud, is caused in the absence of exogenous IAA (Saniewski et al., 2010). Exogenously applied IAA had little or slight inhibitory effect on growth of the IV internode segments (Tab. 6-8). This suggests that relatively higher levels of endogenous auxin are still present in the IV internode, which accounts for the elongation, and that growth of excised IV internode of tulip stems is limited not by IAA but by other factors. In isolated IV internode segments from tulips growing in field conditions, only sucrose at concentrations of 5.0% and 10.0% slightly inhibited growth of IV internode cut below and above node, treated with IAA (Tab. 6-8). The importance of dynamic changes in carbohydrate metabolism for flower stalk elongation in tulips has been reported (Lambrechts and Kolloffel, 1993; Lambrechts et al., 1994; Balk and de Boer, 1999; Ranwala and Miller, 2008). These results suggest that metabolism of sugars readily utilized in elongating cells is involved in stem elongation of tulips.

Effect of soluble sugars on chlorophylls and phenolic compounds in tulip stems

As shown in Fig. 1, the senescence of the isolated stem segments from growing tulips, kept in distilled water proceeded during the incubation. The application of IAA substantially delayed senescence. On the other hand, the application of sucrose together with IAA evidently delayed senescence. Sucrose at all used concentrations substantially increased chlorophyll contents in stem segments treated with IAA in comparison to IAA applied alone (Fig. 1, Tab. 1 and 2).

In isolated IV internode from tulips growing in field conditions, treated with IAA the content of chlorophylls was much higher than those not treated with IAA, whereas growth was only slightly promoted by IAA. Furthermore, sucrose at all concentrations (1.25%, 2.5%, 5.0% and 10.0%) substantially increased chlorophyll content (Tab. 6-8), whereas sucrose at concentrations of 5.0% and 10.0% inhibited growth of IV internode cut below and above node.

The greatly increased chlorophyll content by sucrose in auxin-induced growth of tulip stem segments, may be caused by delaying of senescence, increased chlorophyll biosynthesis or retarded chlorophyll degradation. It is well known that sugars prevent senescence of cut flowers and vegetables. Sucrose improved the postharvest life of cut flowers of *Limonium* (Doi and Reid, 1995), *Liatris* (Han, 1992), *Eustoma grandiflorum* (Cho et al., 2001), and many other species. Sucrose supply increased longevity and inhibited chlorophyll degradation of broccoli (*Brassica oleracea*) branchlets (Irving and Joyce, 1995). Sucrose and glucose at concentration of 0.2 M is well known to stimulate biosynthesis of chlorophyll in excised, starved leaves of bean exposed to red light, whereas sucrose had no stimulatory effect when the cotyledon was left attached to the leaf, indicating that the endogenous carbohydrate level

Table 6. Interaction of IAA and sucrose (S) on the elongation of IV internode cut above node from tulips growing in field conditions and on the chlorophyll content

Treatment	Initial length of IV internode on April 14th [mm]	Length of IV internode on April 23rd [mm]	Increase of growth of IV internode [mm]	The content of chlorophylls (a+b) [µg/cm length] on April 30th
Control (lanolin) − water	80.7 a	126.2 bc	45.5 ab	4.19 a
IAA 0.1% − water	77.5 a	132.5 c	55.0 b	12.12 b
IAA 0.1% − S 10.0%	69.5 a	106.0 a	36.5 a	18.07 c
IAA 0.1% − S 5.0%	67.2 a	116.2 ab	49.0 ab	18.16 c
IAA 0.1% − S 2.5%	67.5 a	124.7 bc	57.2 b	16.07 c
IAA 0.1% − S 1.25%	71.5 a	129.6 bc	58.1 b	17.08 c

Table 7. Interaction of IAA and sucrose (S) on the elongation of IV internode cut above node from tulips growing in field conditions and on the chlorophyll content

Treatment	Initial length of IV internode on April 22nd [mm]	Length of IV internode on May 2nd [mm]	Increase of growth of IV internode [mm]	The content of chlorophylls (a+b) [µg/cm length] on	
				May 5th	May 12th
Control (lanolin) − water	180.8 c	244.9 b	64.1 c	9.10 a	-
IAA 0.1% − water	162.7 abc	210.9 a	48.2 b	10.57 a	6.54 a
IAA 0.1% − S 10.0%	170.8 bc	200.7 a	29.8 a	15.47 b	13.80 c
IAA 0.1% − S 5.0%	170.8 bc	205.7 a	34.9 a	17.87 bc	16.58 d
IAA 0.1% − S 2.5%	145.5 a	192.7 a	47.1 b	19.56 c	14.52 c
IAA 0.1% − S 1.25%	154.8 ab	197.4 a	42.7 b	19.05 c	11.27 b

Table 8. Interaction of IAA and sucrose (S) on the elongation of IV internode cut below node from tulips growing in field conditions and on the chlorophyll content

Treatment	Initial length of IV internode on April 22nd [mm]	Length of internodes on April 30th [mm]	Increase of growth of IV internode [mm]	The content of chlorophylls (a+b) [µg/cm length] on	
				May 5th	May 12th
Control (lanolin) − water	169.4 b	227.2 c	57.9 c	8.71 a	7.73 ab
IAA 0.1% − water	176.7 bc	226.2 c	49.5 b	13.12 b	6.39 a
IAA 0.1% − S 10.0%	182.5 c	214.2 b	31.8 a	21.61 d	11.31 bc
IAA 0.1% − S 5.0%	149.6 a	186.0 a	36.4 a	22.66 d	12.97 c
IAA 0.1% − S 2.5%	180.2 c	227.9 c	47.6 b	18.72 c	21.03 d
IAA 0.1% − S 1.25%	193.1 d	243.9 d	50.8 b	22.76 d	21.50 d

was saturated (Wolff and Price, 1960). An effect of sugars in stimulating chlorophyll formation was also reported in etiolated detached leaves of various species by other authors cited by Wolff and Price (1960).

As mentioned above, sucrose at higher concentrations also caused stiffening of isolated stem segments and reduced infection by pathogens in comparison to IAA treatment only. Higher concentrations of sucrose such as 2.5% and 5.0%, in the presence of IAA, evidently lowered free phenolic compounds content measured in III and IV internodes, whereas effects of sucrose at 10% on phenolic compound has not been determined in this study (Tab. 2). The decrease in endogenous level of free phenolic compounds is possible to increase lignin contents, resulting in cell wall stiffening and preventing pathogen infection in tulip stems.

Morkunas et al. (2005) showed that exogenous sucrose at concentration of 60 mM caused a marked increase in endogenous concentrations of sucrose, glucose and fructose in embryo axes of yellow lupine (*Lupinus luteus* L.) and induced generally higher levels of isoflavone glycosides and free aglicones (genistein, wighteone, luteone). Exogenous sucrose also stimulated the activity of phenylalanine ammonialyase (PAL), an important enzyme initiating phenylpropanoid metabolism. Disease symptoms of yellow lupine embryo axes by *Fusarium oxysporum* f. sp. *lupine* growing in the presence of sucrose have been reported to be less intensive (Morkunas et al., 2002). Morkunas et al. (2005) also suggest that soluble sugars are involved in the mechanism of resistance, as they can stimulate phenylpropanoid metabolism and contribute to the increase in concentration of isoflavonoids which are important elements of the defence system of legumes. Increasing sucrose concentrations promote phenylpropanoid biosynthesis in grapevine cell cultures (Ferri et al., 2011). Gutierrez et al. (1995) showed that detached leaves, leaf disks and cut stems and hypocotyls of sunflower plants accumulated coumarin phytoalexins. The link between sugar signalling and lignification is particularly interesting. Rogers et al. (2005) suggest that sugars are not only essential sources of carbon skeleton for lignin biosynthesis in *Arabidopsis thaliana* but sucrose may indeed function as a signal to enhance the activity of the lignin biosynthetic pathway, through a mechanism that does not involve direct signalling through hexokinase. Judging from the results obtained in this study, together with the facts described above, stiffness of excised tulip stem after treatment with IAA and sucrose might be caused by enhanced phenylpropanoid metabolism induced by sucrose and in consequence increased production of lignin in tissues. Further studies of the effect of sugars on the phenyl propanoid metabolism in tulip stems will be required.

Soluble sugars, especially sucrose, glucose, and fructose play central role in plant structure and metabolism at the cellular and whole-organism level

(Couée et al., 2006). The main role of sugars in metabolism is its function as a carrier of energy, carbon partitioning, and regulator of osmotic potential. They are involved in many other processes and act also as metabolite signalling molecules that activate specific or hormone-crosstalk transduction pathways, resulting in important modifications of gene expression (Smeekens, 2000; Rolland et al., 2002; Gibson, 2005; Koch, 1996). Arru et al. (2008) showed that sucrose at concentrations of range of 15 to 120 mM did not enhance the growth of tomato hypocotyls segments induced by 2,4-D or brassinolide, but sucrose at a concentration of 15 mM or higher in the presence of 2,4-D or brassinolide induced expansin gene *LeEXPA2* transcript, member of a multigene family of extracellular proteins that mediate cell wall extension and relaxation during growth. Cui et al. (2010) showed that high content of sucrose in adventitious root cultures of *Hipericum perforatum* resulted in osmotic stress and, in turn, induced the accumulation of secondary metabolites.

It is well known that starch, fructose polymers and sucrose are the main storage carbohydrates in the bulb scales of tulips (Moe and Wickstrom, 1973; Thompson and Rutherford, 1977). In the tulip leaves, fructose, glucose, sucrose, myo-inositol, stachyose, tuliposides A and B, and traces of arabinose and xylose were found (Rutter et al., 1977). In the flower stem of tulips the occurrence of sucrose, glucose, fructose and starch is well documented (Lambrechts et al., 1994; Ranwala and Miller, 2008). Sucrose plays a particularly important role in plant growth and development, as it is the major form of sugars translocated in plants (Gonzali et al., 2006). In tulip stems, sucrose interacting with auxin has various effects on the regulation of growth, metabolism of chlorophylls and phenol compounds, and others. Multiple effects of sucrose in the presence of IAA might be due to the fact that plants have multiple sugar-response pathways (Gibson, 2005).

REFERENCES

Arru L., Rognoni S. Poggi A. 2008. Effect of sugars on auxin-mediated *LeEXPA2* gene expression. PLANT GROWTH REGUL. 55: 11-20.

Balk P.A., de Boer A.D. 1999. Rapid stalk elongation in tulip (*Tulipa gesneriana* L. cv. Apeldoorn) and the combined action of cold-induced invertase and the water-channel protein γ TIP. PLANTA 209: 346-354.

Banasik L., Saniewski M. 1985. The effect of different auxins on tulip stalk elongation. ACTA HORT. 167: 193-204.

Bruinsma J. 1963. The quantitative analysis of chlorophyll a and b in plant extracts. PHYTOCHEM. PHYTOBIOL. (CHLOR. METABOL. SYMP.) 2: 241-249.

Cho M.-S. Celikel F.G., Dodge L., Reid M.S. 2001. Sucrose enhances the postharvest quality of cut flowers of *Eustoma grandiflorum* (Raf.) Shinn. ACTA HORT. 543: 305-315.

Couée I., Sulmon C., Gonesbet G., El Amrani A. 2006. Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. J. EXPER. BOT. 57: 449-459.

Cui X.-H. Murthy H.N., Wu C.-H., Pack K.-Y 2010. Sucrose-induced osmotic

stress affects biomass, metabolite, and antioxidant levels in root suspension cultivars of *Hypericum perforatum* L. PLANT CELL TISS. ORGAN. CULT. 103: 7-14.

De Hertogh A. 1974. Principles for forcing tulips, hyacinths, daffodils, Easter lilies and Dutch irises. SCIENTIA HORT. 2: 313-355.

Doi M., Reid M.S. 1995. Sucrose improves the postharvest life of cut flowers of a hybrid Limonium. HORTSCIENCE 30: 1058-1060.

Ferri M., Righetti L., Tassoni A. 2011. Increasing sucrose concentrations promote phenylpropanoid biosynthesis in grapevine cell cultures. J. PLANT PHYSIOL. 168: 189-195.

Gibson S.I. 2005. Control of plant development and gene expression by sugar signaling. CURR. OPIN. PLANT BIOL. 8: 93-102.

Gilford J. McD, Rees A.R. 1973. Growth of tulip shoot. SCIENTIA HORT. 1: 143-156.

Gonzali S., Loreti E., Solfanelli C., Novi G., Alpi A., Perata P. 2006. Identification of sugar-modulated genes and evidence for in vivo sugar sensing in Arabidopsis. J. PLANT RES. 119: 115-123.

Gutierrez M.-C., Parry A., Jorrin J., Edwards R. 1995. Abiotic elicitation of coumarin phytoalexins in sunflower. PHYTOCHEMISTRY 38: 1185-1191.

Han S.S. 1992. Role of sucrose in bud development and vase life of cut *Liatris spicata* (L.) Willd. HORTSCIENCE 27: 1198-1200.

Irving D.E., Joyce D.C. 1995. Sucrose supply can increase longevity of broccoli (*Brassica oleracea*) branchlets kept at 22 ^{o}C. PLANT GROWTH REGUL. 17: 251-256.

Kaur C., Kapoor H.C. 2002. Anti-oxidant activity and total phenolic content of some Asian vegetables. INT. J. FOOD SCI. TECHNOL. 37: 153-161.

Koch K.E. 1996. Carbohydrate-modulated gene expression in plants. ANNU. REV. PLANT PHYSIOL. MOL. BIOL. 47: 509-540.

Lambrechts H., Kolloffel C. 1993. Soluble and insoluble invertase activity in elongation *Tulipa gesneriana* flower stalks. PHYSIOL. PLANT. 89: 830-834.

Lambrechts H., Rook F., Kolloffel C. 1994. Carbohydrate status on tulip bulbs during cold-induced flower stalk elongation and flowering. PLANT PHYSIOL. 104: 515-520.

Moe R., Wickstrom A. 1973. The effect of storage temperature on shoot growth, flowering and carbohydrate metabolism in tulip bulbs. PHYSIOL. PLANT. 28: 81-87.

Morkunas I., Kozłowska M., Ratajczak W. 2002. The role of carbohydrates in early metabolic response of germinating lupine seeds to *Fusarium oxysporum* f. sp. lupine. ACTA AGROBOTANICA 55: 247-254.

Morkunas I., Marczak Ł., Stachowiak J., Stobiecki M. 2005. Sucrose-induced lupine defense against *Fusarium oxysporum*. Sucrose-stimulated accumulation of isoflavonoids as a defense response of lupine to *Fusarium oxysporum*. PLANT PHYSIOL. BIOCHEM. 43: 363-373.

Ranwala A.P., Miller W.B. 2008. Gibberellin-mediated changes in carbohydrate metabolism during flower stalk elongation in tulips. PLANT GROWTH REGUL. 55: 241-248.

Rogers L.A., Dubos C., Cullis I.F., Surman C., Poole M., Willment J., Mansfield S.D., Campbell M.M. 2005. Light, the circadian clock, and sugar perception in the control of lignin biosynthesis. J. EXPER. BOT. 56: 1651-1663.

Rolland F., Moore B., Sheen J. 2002. Sugar sensing and signaling in plants. PLANT CELL 14: 185-205.

Rutter J.C., Johnson W.R., Wilmer C.W. 1977. Free sugars and organic acid in the leaves of various plant species and their compartmentation between the tissues. J. EXPER. BOT. 28: 1019-1028.

Saniewski M., de Munk W.J. 1981. Hormonal control of shoot elongation in tulips. SCIENTIA HORT. 5: 363-372.

Saniewski M., Góraj J., Węgrzynowicz-Lesiak E., Okubo H., Miyamoto K., Ueda J. 2010. Different growth of excised and intact fourth internode after removal of the flower bud in growing tulips: Focus and auxin action. J. FRUIT ORNAM. PLANT RES. 18: 297-308.

Saniewski M., Okubo H., Miyamoto K., Ueda J. 2005. Auxin induces growth of stem excised from growing shoot of cooled tulip bulbs. J. FAC. AGRIC., KYUSHU UNIV. 50: 481-488.

Saniewski M., Okubo H., Miyamoto K., 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.

Singleton V.L., Rossi J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. AM. J. ENOL. VITICULTURE 16: 144-158.

Smeekens S. 2000. Sugar-induced signal transduction in plants. ANNU. REV. PLANT PHYSIOL. MOL. BIOL. 51: 49-81.

Thompson T., Rutherford P.P. 1977. Morphological development and carbohydrates changes of forced tulips. J. HORT. SCI. 52: 9-17.

Wolff J.B., Price L. 1960. The effect of sugars on chlorophyll biosynthesis in higher plants. J. BIOL. CHEM. 235: 1603-1608.

WPŁYW CUKRÓW NA WZROST I ZAWARTOŚĆ CHLOROFILU W IZOLOWANYCH ŁODYGACH TULIPANA W OBECNOŚCI KWASU INDOLILO-3-OCTOWEGO

Elżbieta Węgrzynowicz-Lesiak, Marian Saniewski, Justyna Góraj, Marcin Horbowicz, Kensuke Miyamoto i Junichi Ueda

S T R E S Z C Z E N I E

Celem badań było poznanie wpływu sacharozy i glukozy na indukowany przez auksynę (IAA) wzrost izolowanych łodyg z rosnących tulipanów i izolowanych bezpośrednio z przechłodzonych i nieprzechłodzonych cebul tulipana, oraz na wzrost izolowanego IV międzywęźla z rosnących roślin w obecności auksyny. We wszystkich traktowaniach usunięty pąk kwiatowy był zastąpiony przez IAA (0,1%, w paście lanolinowej), dolna część izolowanych segmentów była trzymana w wodzie destylo-

wanej lub w roztworze cukrów o różnym stężeniu. Indukowany przez IAA wzrost izolowanych łodyg z rosnących tulipanów był hamowany przez sacharozę w stężeniu 5% i 10%, a sacharoza w stężeniu 1,25% i 2,5% nie powodowała zmian we wzroście. Sacharoza we wszystkich zastosowanych stężeniach opóźniała starzenie się łodyg i powodowała wzrost zawartości chlorofilu w izolowanych łodygach w obecności IAA. Sacharoza indukowała większą sztywność izolowanych łodyg w obecności IAA, a łodygi były dużo bardziej odporne na infekcje przez grzyby w porównaniu z izolowanymi łodygami traktowanymi tylko IAA. Mannitol i sorbitol w stężeniach 5% i 10% hamowały wzrost izolowanych łodyg indukowany przez auksynę. Łodygi tulipana izolowane bezpośrednio z przechłodzonych i nieprzechłodzonych cebul były bardziej wrażliwe na traktowanie sacharozą i glukozą w obecności auksyny niż łodygi izolowane z rosnących tulipanów; stwierdzono większe hamujące działanie cukrów. Sacharoza w stężeniach 5% i 10% w małym stopniu hamowała wzrost IV międzywęźla traktowanego IAA, ale wszystkie zastosowane stężenia sacharozy (1,25%; 2,5%; 5% i 10%) powodowały wzrost zawartości chlorofilu. W pracy dyskutowane jest możliwe działanie sacharozy w interakcji z auksyną w regulacji wzrostu izolowanych łodyg, chociaż mechanizm ten jest złożony ze względu na wielokierunkowe oddziaływanie cukrów na metabolizm rośliny.

Słowa kluczowe: kwas indolilo-3-octowy (IAA), eksplantaty łodygi, tulipany, wzrost, sacharoza, glukoza, chlorofil