# STIMULATORY EFFECT OF 2,3,5-TRIIODOBENZOIC ACID (TIBA) ON SHOOT GROWTH AND FLOWERING OF PARTIALLY COOLED TULIP (*Tulipa gesneriana* L.) BULBS

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A B S T R A C T

In the present work, 2,3,5-triiodobenzoic acid (TIBA) was applied to uncooled tulip bulbs, cultivars Apeldoorn and Gudoshnik, before flower bud formation, at the beginning of July and after flower bud formation, in October and November. Shoot growth and flowering of partially dry-cooled bulbs were substantially stimulated. These results strongly suggest that TIBA partially replaces the cold requirement of the tulip bulbs. In addition, the effect of TIBA is similar to gibberellins applied exogenously to the bulbs. Such a gibberellin application partially substitutes for cold treatment. Gibberellin application stimulates shoot growth and flowering of tulips. The mode of action of TIBA is discussed in relation to auxin action in tulips.

**Key words**: bulbs, shoot growth, flowering, tulip, cold treatment, 2,3,5-triiodobenzoic acid (TIBA)

## 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. Such a requirement indicates that tulip bulbs have a kind of dormancy released by exposure to low temperature (Kamerbeek et al., 1972; De Hertogh, 1974). 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 the 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). Exogenously applied gibberellins partially substituted for cold treatment of tulip bulbs. Gibberellin application stimulated shoot growth and flowering (Van Bragt and Van Ast, 1976; Van Bragt and Zijlstra, 1971; Rudnicki et al., 1976; Jones and Hanks, 1984; Hanks, 1984, 1985).

It is also well-known that auxins can play an important role in the growth and development of tulips. Inhibition of tulip stem growth in fully cooled tulip bulbs after excision of all leaves and flower bud, as a source of auxins, was fully recovered after application of auxin at the cut surface of the top internode (Op den Kelder et al., 1971; Hanks and Rees, 1977; Okubo and Uemoto, 1985; Okubo et al., 1986; Saniewski and deMunk, 1981; Banasik and Saniewski, 1985). Removal of exogenous auxin in different stages of tulip stem growth almost totally stopped further elongation of all internodes (Saniewski and Węgrzynowicz-Lesiak, 1993). Also, application of IAA as a lanolin paste, to the cut surface of the top internode of tulip shoot excised from cooled bulbs and/or from growing shoots in cooled bulbs, after removal of flower bud and all leaves, promoted the extreme growth of all internodes. While the growth of all internodes treated only with lanolin in the same way as the IAA application, was very small (Saniewski et al., 2005, 2007). Thus, a continuous supply of auxin is necessary for tulip stem growth. Together with the fact mentioned above, the elongation of the all internodes in tulips has been suggested to be substantially regulated by the interaction of auxins with gibberellins (Okubo and Uemoto, 1985; Okubo et al., 1986; Saniewski, 1989; Saniewski and Kawa-Miszczak, 1992; Rietveld et al., 2000).

The crucial role of auxin in tulip stem growth, was confirmed in studies using 2,3,5-triiodobenzoic acid (TIBA), well known for many years an inhibitor of the basipetal polar auxin transport in plants (Niedergang-Kamien and Leopold, 1957; Morris et al., 1973). Okubo and Uemoto (1985) showed that TIBA treatment at the first internode of sprouting tulip shoot inhibited the dark-induced elongation of the first internode. Such a treatment also decreased the amount of diffusible auxin from the upper organs into the first internode but did not affect the gibberellin amount. Saniewski and Okubo (1997) found that IAA applied in the place of the removed flower bud and after the excision of leaves, promoted flower stalk elongation in the non precooled and precooled, rooted and derooted tulip bulbs. They also found that TIBA applied in the middle of the $4^{th}$ internode (below IAA application) greatly inhibited the growth of lower internodes. More detail studies about the effect of TIBA on stem growth induced by IAA and NAA in precooled rooted tulip bulbs were presented by Saniewski and Okubo (1998); TIBA is effective in inhibiting either IAA- and NAA-induced elongation, below or above the point of treatment.

Recently, the effects of TIBA with gibberellic acid ($GA_3$) and root excision on growth and flowering of a few cultivars of non pre-cooled tulip bulbs were investigated (Geng et al., 2005ab). In these experiments the bulbs were put on Petri dishes with distilled water, $GA_3$ or $GA_3$ solution after lanolin paste containing 0.5% TIBA was smeared around the base of the outer scale near the rim of the root primordia of the bulbs with or without excision of the root primordia. $GA_3$ partly replaced the cold requirement of the bulbs, and when TIBA was applied with $GA_3$, the growth and flowering were promoted even more. The endogenous content of IAA in the basal plate and lower internodes was higher with $GA_3$ + TIBA treatment than $GA_3$ treatment alone. Such results indicate that TIBA blocked the auxin transport at the basal plate and increased auxin content in the stems. Such action promoted early flowering and internode elongation.

Saniewski et al. (2011) in previous preliminary studies, showed that application of TIBA to the partially dry-cooled tulip bulbs substantially promoted shoot growth and flowering. In the present study, we report further new data on the stimulatory effect of TIBA on growth and flowering of partially cooled tulip bulbs.

## MATERIAL AND METHODS

Bulbs of tulip (*Tulipa gesneriana* L.) cultivars Apeldoorn and Gudoshnik with circumferences of 10-12 cm, were purchased from commercial stocks. After lifting, the bulbs were stored at 18-22 °C. Dry scales were then removed. Next, lanolin alone or 2.3.5-triiodobenzoic acid (TIBA) at a concentration of 0.2, and 0.5% in lanolin, was applied around the basal plate of the scale (about 1.2 cm width). As an additional control, intact bulbs with dry scales were also used. The following experiments with tulip bulbs in the successive years were made:

- Treatments of TIBA 0.5% in tulip bulbs ‘Apeldoorn’ made on November 17, and directly moved for dry-cooling at 5 °C, and planted in a greenhouse on January 21.
- Treatments of TIBA 0.2 and 0.5% in tulip bulbs ‘Apeldoorn’ and ‘Gudoshnik’ made on October 19, and directly moved for dry-cooling at 5 °C, and planted in a greenhouse after a different period of cooling (55, 69, 74 and 76 days).

- Treatments of TIBA 0.2 and 0.5% in tulip bulbs 'Apeldoorn' made on October 20, and directly moved for dry-cooling at 5 $^{o}$ C and planted in a greenhouse in December.
- Treatment of TIBA 0.2 and 0.5% in tulip bulbs 'Apeldoorn' made on July 7 and on October 3, and moved for dry-cooling on October 12, and planted in a greenhouse after a different period of cooling (48, 55, 62 and 68 days).

After cooling, the bulbs of all treatments were planted in a greenhouse at a temperature of 17-20 $^{o}$C under natural light conditions. Length of different internodes or only the total length of the stem was measured during the duration of the experiment. Flowering time was recorded and plants were photographed. For each treatment 15 bulbs were used. Data were subjected to an analysis of variance and evaluated introducing the Duncan's multiple range tests at a 5% level of significance.

## RESULTS AND DISCUSSION

Studies were carried out for three consecutive years. During that time, 2,3,5-triiodobenzoic acid (TIBA) was applied to uncooled tulip bulbs 'Apeldoorn' and 'Gudoshnik', after flower bud formation, in October and November, and then the tulip bulbs were partially dry-cooled. Our studies showed that this procedure substantially stimulated shoot growth and flowering (Tab. 1-4, Figs. 1-4). It should be mentioned, that TIBA did not stimulate shoot growth and flowering in uncooled tulip bulbs, without partial dry-cooling (data not presented). This effect of TIBA was weak in fully cooled tulip bulbs. Flowering of tulips in natural conditions is strictly connected with shoot growth.

TIBA applied before flower bud formation (July) on the bulbs 'Gudoshnik', did not affect flower bud development and after partial cooling of the bulbs stimulated shoot growth and flowering (Tab. 4, Fig. 4).

Table1. The effect of TIBA treatment of uncooled tulip bulbs 'Apeldoorn', which were then dry-cooled at 5 $^{o}$C, on shoot growth and flowering. Treatments were made directly before cooling on November 17 and on January 21 the bulbs were planted in a greenhouse. There were 15 bulbs per treatment used

| Treatments | Length of internodes [cm] on Feb. 26 | | | | | Flowering time |
|---|---|---|---|---|---|---|
| | 1st | 2nd | 3$^{rd}$ | 4th | total | |
| Control intact | 2.6 b | 3.0 a | 4.6 b | 6.2 a | 16.4 a | March 2 |
| Control lanolin | 1.9 a | 2.7 a | 3.4 a | 6.4 a | 14.4 a | March 2 |
| TIBA 0.5% | 2.7 b | 4.0 b | 6.4 c | 16.0 b | 29.0 b | February 22 |

Table 2. The effect of TIBA applied before cooling of tulip bulbs 'Apeldoorn 'and 'Gudoshnik', which were then dry-cooled at 5 °C by different periods, on shoot growth and flowering. Cooling was started on October 19. Flowering time is indicated in parentheses

| Time of planting (days of cooling) | Cultivar | Date of measurement of stem length | Length of stem [cm] | |
|---|---|---|---|---|
| | | | Control | TIBA 0.5% |
| Dec. 14 (55 days) | Gudoshnik | Jan. 31 | 10.0 a (Feb. 4) | 45.0 b (Jan. 31) |
| | Apeldoorn | Jan. 31 | 17.0 a (Feb. 4) | 36.5 b (Jan. 31) |
| Dec. 28 (69 days) | Gudoshnik | Jan. 31 | 24.5 a (Feb. 6) | 42.5 b (Feb. 2) |
| | Apeldoorn | Jan. 31 | 23.0 a (Feb. 6) | 34.5 b (Feb. 2) |
| Jan. 2 (74 days) | Gudoshnik | Jan. 31 | 36.5 a (Feb. 1) | 49.0 b (Jan. 31) |
| | | Feb. 12 (after flowering) | 50.6 a | 59.0 b |
| | Apeldoorn | Jan. 31 | 25.5 a (Feb. 2) | 38.0 b (Jan. 31) |
| | | Feb. 12 (after flowering) | 46.7 a | 51.4 b |
| Jan. 4 (76 days) | Gudoshnik | Jan. 31 | 33.5 a (Feb. 3) | 53.0 b (Jan. 31) |
| | | Feb. 12 (after flowering) | 52.0 a | 61.9 b |
| | Apeldoorn | Jan. 31 | 28.0 a (Feb. 3) | 37.5 b (Jan. 31) |
| | | Feb. 12 (after flowering) | 44.4 a | 51.5 b |

Table 3. The effect of TIBA treatment of tulip bulbs 'Apeldoorn' on July 17, on tulip shoot growth and flowering after cooling of bulbs from October 20 until time of planting on December 6

| Treatments | Length of stem [cm] | | Number of days from planting until flowering and date of flowering (in parentheses) |
|---|---|---|---|
| | Jan. 14 | Jan. 18 | |
| Control (without scales) | 3.9 | 10.7 | 44 (Jan. 19) |
| Control (lanolin) | 8.8 | 18.3 | 40 (Jan. 15) |
| TIBA 0.2% | 25.9 | 30.9 | 34 (Jan. 9) |
| TIBA 0.5% | 25.0 | 28.3 | 33 (Jan. 8) |

Thus, TIBA partially replaced the cold requirement of the tulip bulbs. The effect of TIBA is similar to exogenously applied gibberellins to bulbs. Such a procedure partially substituted for the cold treatment, and stimulated shoot growth and flowering of tulips (Van Bragt and Zijstra, 1971; Van Bragt and Van Ast, 1976; Rudnicki et al., 1976; Jones

**Figure 1**. Stimulatory effect of TIBA 0.5% treatment of uncooled tulip bulbs ‘Apeldoorn’ which were then dry-cooled at 5 °C, on shoot growth and flowering. Treatments were made directly before cooling on November 17 and on January 21. The bulbs were planted in a greenhouse (see Table 1); photographed on Feb. 22 (the left picture) and on Feb. 26 (the right picture)

**Figure 2**. Stimulatory effect of TIBA 0.5% on shoot growth and flowering of tulips when applied to uncooled bulbs ‘Gudoshnik’ and ‘Apeldoorn’ on October 19, which were directly moved for dry-cooling and planted in a greenhouse on December 14 (55 days of cooling) (see Table 2); photographed on January 24 (the left picture) and on January 31 (the right picture)
on left – cv. Gudoshnik; control and TIBA 0.5%, respectively
on right – cv. Apeldoorn; control and TIBA 0.5%, respectively

and Hanks, 1984; Hanks, 1984, 1985). As previously suggested by Geng et al. (2005 a,b), TIBA stimulated shoot growth and flowering through blocking auxin polar transport from pistil and leaves to the basal plate and this, accumulation of auxin takes place in the tulip shoots.

Questions arise about just what is the role of TIBA as a replacement for the partial cooling of tulip bulbs. There are also questions about how the action of TIBA to gibberellins has a stimulatory effect on shoot growth and flowering of partially cooled tulip bulbs. One possibility is that the accumulated auxin in tulip shoot, as a result of TIBA action, directly stimulated shoot growth and flowering. Another hypothesis is that

**Figure 3**. The effect of TIBA treatment of tulip bulbs 'Apeldoorn' on July 17 on tulip shoot growth and flowering after cooling of bulbs from October 20 until the time of planting on December 6; photographed on January 7 (the left picture) and on January 12 (the right picture); from left to right: control (intact bulbs – with dry scales), control, TIBA 0.2%, TIBA 0.5%

**Figure 4**. The effect of TIBA treatment of tulip bulbs on July 7 (the left picture) and on October 3 (the right picture) on tulip stem growth and development, after dry-cooling from October 12 to December 1 (48 days of cooling); photographed on January 3; from left to right: control (intact bulbs – with dry scales), control, TIBA 0.2%, TIBA 0.5%

the accumulated auxin in tulip shoot induces gibberellins biosynthesis. The interaction of auxin with gibberellins is responsible for tulip shoot growth and flowering.

Kawa and Saniewski (1986) showed that gibberellic acid ($GA_3$) had a strong stimulatory effect in increasing the length and fresh weight of pistils isolated from uncooled tulip bulbs, but to lesser degree in the case of cooled bulbs, cultured *in vitro*. Thus, it is probable that gibberellins produced during cooling stimulate flower bud development – especially pistil growth, as a source of auxin. Saniewski (1989) also suggested that gibberellins produced during the cooling of bulbs play an important role in the flower bud development. He also suggested that other gibberellins are synthesized during shoot growth as result of auxin action and together with auxin, control the stem elongation in tulips. Recently, auxin has substantially induced gibberellins

Table 4. The effect of TIBA treatment of tulip bulbs on July 7 and October 3, on tulip growth and development after different periods of cooling; cooling started on October 12

| Date of planting/ treatment | Treatment on July 7 | | | Treatment on October 3 | | Days from planting to flowering /date of flowering (in parentheses) |
|---|---|---|---|---|---|---|
| | Date of measurements of stem length [cm] | | Days from planting to flowering /date of flowering (in brackets) | Date of measurements of stem length [cm] | | |
| *Planting on December 1 (48 days of cooling)* | Jan. 20 | Jan. 24 | | Jan. 20 | Jan. 24 | |
| Control – intact | 15.6 a | 21.6 a | 49 c (Jan. 19) | 17.8 a | 21.1 a | 48 b (Jan. 18) |
| Control – lanolin | 15.4 a | 19.8 a | 50 c (Jan. 20) | 18.0 a | 23.7 a | 47 b (Jan. 17) |
| TIBA 0.2% | 25.9 b | 27.3 b | 40 b (Jan. 10) | 31.0 b | 31.2 b | 37 a (Jan. 7) |
| TIBA 0.5% | 31.0 c | 31.6 b | 32 a (Jan. 2) | 30.0 b | 30.5 b | 36 a (Jan. 6) |
| *Planting on December 8 (55 days of cooling)* | Jan. 20 | Jan. 24 | | Jan. 20 | Jan. 24 | |
| Control – intact | 19.2 a | 24.7 a | 41 c (Jan. 18) | 15.8 a | 20.5 a | 42 c (Jan. 19) |
| Control – lanolin | 21.8 a | 28.4 b | 40 c (Jan. 17) | 15.0 a | 19.7 a | 43 c (Jan. 20) |
| TIBA 0.2% | 29.1 b | 30.7 b | 32 b (Jan. 9) | 29.4 b | 31.4 b | 36 b (Jan. 13) |
| TIBA 0.5% | 28.5 b | 30.0 b | 28 a (Jan. 5) | 30.3 b | 31.8 b | 33 a (Jan. 10) |
| *Planting on December 15 (62 days of cooling)* | Jan. 20 | Jan. 24 | | Jan. 20 | Jan. 24 | |
| Control – intact | 19.3 a | 25.1 a | 36 b (Jan. 20) | 19.4 a | 23.0 a | 36 c (Jan. 20) |
| Control – lanolin | 20.6 a | 26.4 a | 35 b (Jan. 19) | 21.0 a | 25.8 b | 36 c (Jan. 20) |
| TIBA 0.2% | 33.8 b | 35.3 b | 28 a (Jan. 12) | 34.0 c | 36.1 c | 28 a (Jan. 12) |
| TIBA 0.5% | 33.9 b | 35.5 b | 27 a (Jan. 11) | 30.3 c | 34.0 c | 31 b (Jan. 15) |
| *Planting on December 21 (68 days of cooling)* | Jan. 20 | Jan. 24 | | Jan. 20 | Jan. 24 | |
| Control – intact | 19.1 a | 22.5 a | 32 c (Jan. 22) | 17.6 a | 21.5 a | 32 b (Jan. 22) |
| Control – lanolin | 19.4 a | 23.2 a | 33 c (Jan. 23) | 18.7 a | 23.5 a | 32 b (Jan. 22) |
| TIBA 0.2% | 27.3 b | 33.6 b | 27 b (Jan. 17) | 26.1 b | 31.4 b | 29 a (Jan. 19) |
| TIBA 0.5% | 31.5 c | 34.7 b | 25 a (Jan. 15) | 26.4 b | 30.2 b | 28 a (Jan.18) |

in a range of plant species (Ross et al., 2000, 2003; Ross and O'Neill, 2001; Wolbang et al., 2004; Wolbang and Ross, 2001; O'Neill and Ross, 2002; Frigerio et al., 2006). In addition, it is probable that IAA affects levels of bio-active GAs in tulip shoots.

We suggest that the physiological effect of TIBA, similar to gibberellins, depends on the direction from which it approaches the target cells as well as on its biochemical involvement. In fact, this action is connected with regulating the accumulation of auxin in tulips. The presence of TIBA as an inhibitor of polar auxin transport would affect a suitable hormonal balance in favor of the earlier flowering of partially cooled tulip bulbs.

IAA has been well known to be metabolized in plant tissues. IAA might be decomposed or conjugated to other metabolites. Earlier, we have examined the distribution of radioactivity in different tulip parts and organs treated with labeled [1-$^{14}$C] IAA on the top of the last internode after the removal of all leaves and the flower bud, as related to stem growth (Banasik et al., 1985). Radioactivity was detected along the entire stem and in the basal plate. There were only traces of radioactivity found in the scales of the mother bulb and in the newly developing bulblets. It is interesting that the highest intensity of radioactivity was found in the upper part of the stem, then it gradually decreased towards the base of the stem. However, until now, we have not identified the radioactive constituents present in the stem and in the basal plate. It is probable, that IAA or its metabolites control enzymatic mobilization of storage carbohydrates in the scales by producing the factor(s) in the basal plate which is/are transferred to the scales and stimulate(s) or induce(s) enzyme activities.

In conclusion, the presence of TIBA as an inhibitor of polar auxin transport, would affect a suitable hormonal balance in favor of the earlier flowering of partially cooled tulip bulbs. Further studies on the effect of TIBA on endogenous levels of auxins and bio-active gibberellins will be required.

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# STYMULUJĄCY WPŁYW KWASU 2,3,5-TRIJODOBENZOESOWEGO (TIBA) NA WZROST PĘDU I KWITNIENIE CZĘŚCIOWO PRZECHŁODZONYCH CEBUL TULIPANA (*Tulipa gesneriana* L.)

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

Kwas 2,3,5-trijodobenzoesowy (TIBA) jest dobrze znany od wielu lat jako inhibitor bazypetalny polarnego transportu auksyny w roślinach. W poprzednich naszych badaniach wykazano, że TIBA hamuje wzrost łodygi tulipana indukowany przez

auksynę w cebulach w pełni przechłodzonych, a łączne traktowanie nieprzechłodzonych cebul tulipana TIBA z kwasem giberelinowym (GA), po usunięciu korzeni, przyspiesza wzrost pędu i kwitnienie w porównaniu z traktowaniem samym GA. Stwierdzono, że traktowanie nieprzechłodzonych cebul tulipana TIBA powodowało stymulujący wpływ na wzrost pędu i kwitnienie częściowo przechłodzonych cebul w 5 °C.

W obecnych badaniach udokumentowano, że traktowanie cebul tulipana ‘Apeldoorn’ i ‘Gudoshnik’ TIBA w stężeniu 0.2% i 0.5% w paście lanolinowej na początku lipca (przed utworzeniem pąka kwiatowego) i w październiku i listopadzie (po utworzeniu pąka kwiatowego) przyspiesza wzrost pędu i kwitnienie częściowo przechłodzonych cebul w 5 °C. Wyniki te sugerują, że TIBA zastępuje chłodzenie cebul i oddziałuje podobnie jak gibereliny, które zastępują efekt chłodzenia u częściowo przechłodzonych cebul tulipana.

Wydaje się prawdopodobne, że stymulujący wpływ TIBA na wzrost pędu i kwitnienie tulipanów jest spowodowany przez blokowanie polarnego transportu auksyny ze słupka i liści do piętki i stąd następuje akumulacja auksyny w pędach jak wcześniej sugerowano (Geng i in., 2005 a,b), a auksyna może wpływać na podwyższenie endogennego poziomu giberelin.

**Słowa kluczowe**: cebule, wzrost pędu, kwitnienie, tulipan, traktowanie chłodem, kwas 2,3,5-trijodobenzoesowy (TIBA)