J. Fac. Agr., Kyushu Univ., **43** (3·4), 411–418 (1999)

Gibberellin Induces Shoot Growth and Flowering in Nonprecooled Derooted Bulbs of Tulip (*Tulipa gesneriana* L.)

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(Received October 30, 1998 and accepted November 6, 1998)

Effect of gibberellic acid (GA_3) at a concentration of 200 mgl^{-1} on shoot growth and flowering in nonprecooled derooted tulip bulbs cvs. Gudoshnik and Apeldoorn was investigated. In control nonprecooled derooted bulbs flower bud blasting occurred and no growth of stem was observed. GA_3 greatly induced shoot growth and flowering. The stimulatory effect of gibberellin on shoot growth and flowering in nonprecooled derooted bulbs was much stronger than in case of nonprecooled rooted bulbs (previously published results). GA_3 stimulated also stem growth after excision of flower bud with upper part of leaves and scales. It is probable that excision of all roots in nonprecooled bulbs decreases the level of abscisic acid and exogenous gibberellic acid is more effective in comparison to nonprecooled rooted bulbs. Or it is also possible that better penetration and uptake of gibberellin take place after removal of roots. It seems that gibberellin can substitute for cold requirement and that interaction of exogenous gibberellin with endogenous auxin controls stem growth.

INTRODUCTION

Tulip bulbs, with terminal buds containing a complete flower, require a period of 12–16 weeks of low temperature treatment for floral stalk elongation (De Hertogh, 1974). It is well known that during the cooling of the tulip bulbs the amount of free gibberellins increases (Aung and De Hertogh, 1967; 1968; De Hertogh *et al.*, 1971; Van Bragt, 1971; Aung and Rees, 1974; Hanks and Rees, 1980). Hanks and Rees (1980) found two peaks of activity of gibberellin-like substances in tulips sampled at intervals from October (planting time) until the following April (flowering time). The first one occurred in December or in early-January samples, before the cold requirement was completed, and the second peak occurred around the time of rapid shoot extension and flowering found in roots, scales, leaves, stems, daughter bulbs and in flowers. Recently, however, 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.

Saniewski and De Munk (1981) and Banasik and Saniewski (1985) concluded that elongation of all internodes in cooled tulip bulbs is promoted by auxin produced in leaves and flower buds, and that the auxin-release or response system was stimulated by

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gibberellins. Recently it has been suggested that the elongation of all internodes in tulips is controlled by auxin and gibberellins (Okubo and Uemoto, 1985; 1986; Okubo *et al.*, 1986; Saniewski, 1989). Okubo and Uemoto (1986) suggested that two different gibberellins are involved in controlling the elongation of the lower and upper internodes of tulip flower stalk. Kawa and Saniewski (1986) showed that gibberellic acid had a strong stimulatory effect in increasing the length and fresh weight of pistil isolated from uncooled tulip bulbs but to lesser degree in the case of cooled bulbs, cultured in vitro. Saniewski (1989) suggested that gibberellins produced during the cooling of bulbs play an important role in the flower bud development, mostly pistil growth, and other gibberellins are synthesized during shoot growth and, together with auxin, control the stem elongation in tulips. Rebers *et al.* (1995) also suggested that in tulip GAs are involved in the stalk elongation response.

Exogenously applied gibberellins cannot substitute for cold treatment of uncooled tulip bulbs but do for only partially cooled bulbs and stimulate shoot growth and flowering (Van Bragt and Zijlstra, 1971; Rudnicki *et al.*, 1976; Van Bragt and Van Ast, 1976; Cocozza Talia and Stellacci, 1979; Hanks, 1984; 1985; Jones and Hanks, 1984).

In the present work the stimulatory effect of gibberellin on tulip shoot growth and flowering in nonprecooled derooted tulip bulbs is documented.

MATERIALS AND METHODS

Plant materials

Tulip bulbs cvs. Gudoshnik and Apeldoorn, with a circumference of 10–11 cm, after lifting were stored at 18–20 °C until used for experiments between November 10–22 (nonprecooled bulbs). After the removal of dry scales the following experiments were conducted.

Experiment 1

All roots were excised and bulbs were kept on petri dishes 1) with distilled water or 2) with gibberellic acid (GA_3) at a concentration 200 mgl^{-1}. In a second group all roots were excised and the upper part of the scales, together with flower bud and the upper part of the leaves at the level of the flower bud, were cut and the bulbs were kept on petri dishes 3) with distilled water or 4) with GA_3 at a concentration of 200 mgl^{-1}.

Experiment 2

All roots were excised and the apices of sprouted leaves were treated as follows: a) untreated, b) lanolin on leaf apex, c) IAA 0.1% in lanolin on leaf apex, d) lanolin after removal of 2 mm leaf apex, or e) IAA 0.1% in lanolin after removal of 2 mm leaf apex. The treated bulbs were kept on petri dishes 1) with distilled water or 2) with GA_3 at a concentration of 200 mgl^{-1}.

In both experiments 15 bulbs per treatment were used and every experiment was repeated twice. Newly appeared roots were removed daily. During the experiments the shoot growth and flowering were measured and photographed.

Table 1. Effect of GA_3 200 mgl^{-1} on shoot growth and flowering of nonprecooled derooted bulbs.

Treatment	Length of sprout (first leaf) or *stem with flower bud (mm)					Length of internode (mm) on Dec. 22				
	Nov. 11	Nov. 30	Dec. 3	Dec. 9	Dec. 22	1st	2nd	3rd	4th	Total
Roots removed										
cv. Gudoshnik										
water	23.3	34.9	46.4	64.7	81.5		–[a]			
GA_3	28.1	51.2	71.1	118.4	*243.4	32.0	25.9	30.9	86.5	175.3
cv. Apeldoorn										
water	26.9	39.1	48.3	60.9	73.6		–[a]			
GA_3	31.1	50.5	66.2	98.1	*215.7	14.1	17.9	27.2	95.2	154.4
Roots removed and flower bud together with upper part of leaves and scales excised										
cv. Gudoshnik										
water	7.1	10.8	15.6	27.9	37.0		–[b]			
GA_3	8.3	17.4	29.6	33.7	36.8	31.3	16.5	7.4	11.1	66.3
cv. Apeldoorn										
water	6.5	10.8	16.6	23.5	30.0		–[b]			
GA_3	9.4	18.5	30.2	34.5	37.0	26.5	16.7	11.4	11.7	66.3

[a]No stem growth, and flower bud blasting.
[b]No stem growth.

RESULTS AND DISCUSSION

Experiment 1

The shoot growth of nonprecooled derooted tulip bulbs was very small in both cultivars, only sprouting of leaves was observed and flower bud blasting took place (Table 1, Fig. 1). Gibberellic acid at a concentration of 200 mgl^{-1} caused the breaking dormancy and induced the shoot growth and flowering in nonprecooled derooted tulip bulbs. GA_3 also stimulated stem growth after excision of the flower bud and the upper part of the leaves at the level of the flower bud. Thus, independent of the presence or absence of a flower bud in nonprecooled derooted tulip bulbs gibberellin stimulated stem growth, but was much less effective in cases where flower bud and upper part of leaves were excised. It seems that exogenously applied gibberellin stimulates pistil growth, and interaction of exogenous gibberellin with endogenous auxin is responsible for elongational growth of stem.

Experiment 2

IAA 0.1% applied on leaf apex did not affect shoot growth of nonprecooled tulip bulbs kept on the water or treated with GA_3 (Table 2, Fig. 2). It was suggested previously that the elongation of all internodes in precooled tulip bulbs is controlled by interaction of auxin and gibberellin (Okubo and Uemoto, 1985; 1986; Okubo *et al.*, 1986; Saniewski, 1989). The stimulatory effect of gibberellin on shoot growth in nonprecooled derooted

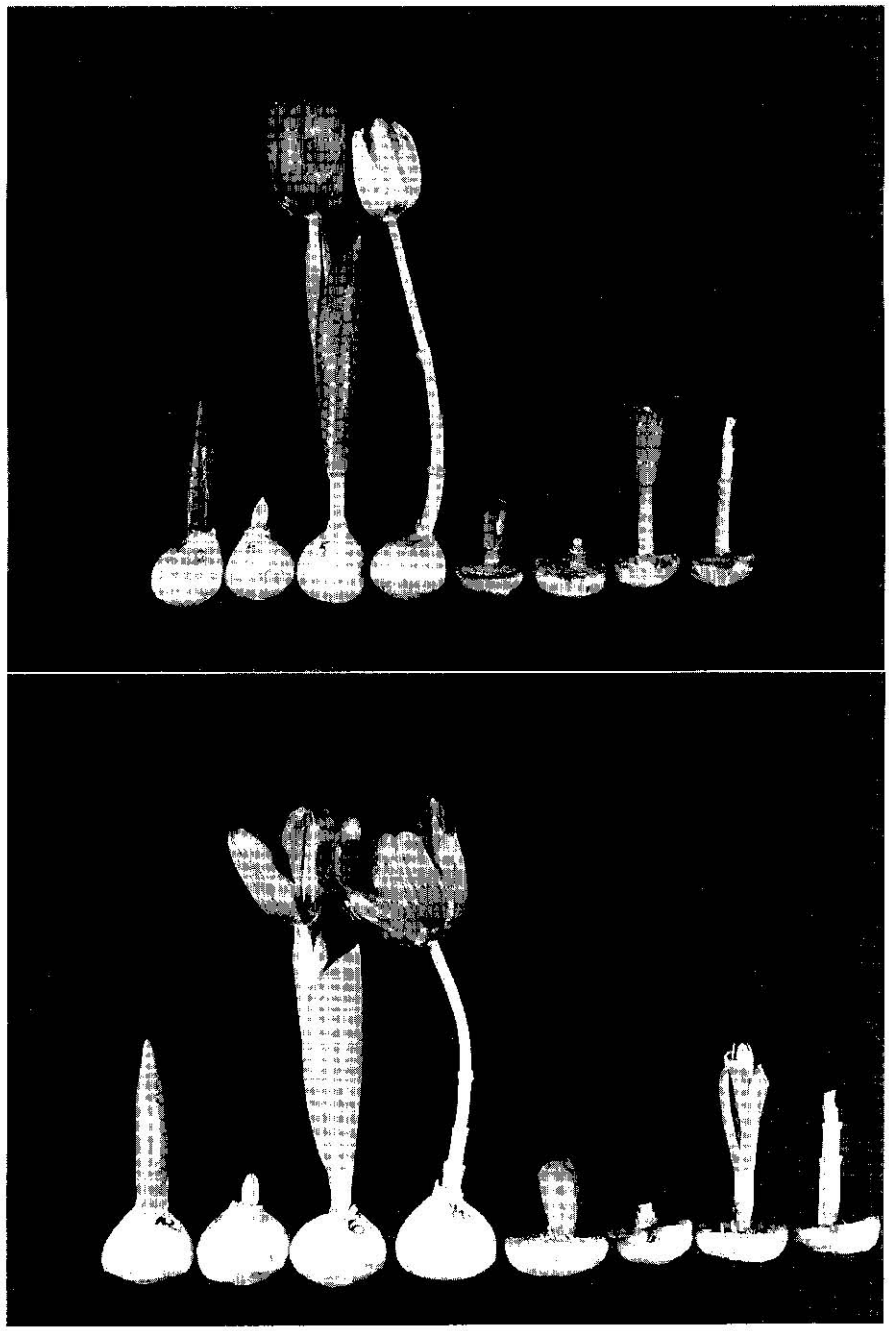

Fig. 1. Effect of GA_3 200 mgl^{-1} on shoot growth and flowering of nonprecooled derooted bulbs. Upper; cv. Gudoshnik, lower; cv. Apeldoorn. Treatment was on November 22 and the plants were photographed on December 17, either with leaves (Left) or after removal of all leaves (Right) before photography. From left to right, control, distilled water–no growth of stem and flower bud blasting can be observed, GA_3–stimulatory effect on shoot growth and flowering, control, distilled water–small growth of leaves and stem after cut of flower bud together with leaves and scales, GA_3–stimulatory effect on stem growth can be observed.

Table 2. Effect of IAA 0.1% treatment on the leaf apex on shoot growth and flowering of nonprecooled derooted bulbs cv. Gudoshnik kept in water and GA_3 200 mgl^{-1}.

Treatment	Length of sprout (first leaf) or *stem with flower bud (mm)			Length of internode (mm) on Dec. 12				
	Dec.2	Dec.8	Dec.12	1st	2nd	3rd	4th	Total
Untreated								
water	43.7	57.5	75.7		–[a]			
GA_3	66.9	93.4	*205.0	19.5	18.6	20.9	75.8	134.8
Lanolin on intact leaf apex								
water	49.4	63.8	84.1		–			
GA_3	69.5	98.9	*229.1	25.6	22.0	26.3	83.9	157.8
IAA 0.1% on intact leaf apex								
water	63.1	82.7	103.5		–			
GA_3	82.6	117.6	*180.9	24.5	18.6	18.4	62.5	124.0
Lanolin after removal of 2 mm leaf apex								
water	43.8	57.9	75.9		–			
GA_3	60.5	87.6	*232.5	31.8	23.6	26.8	86.6	168.8
IAA 0.1% after removal of 2 mm leaf apex								
water	56.3	77.9	99.3		–			
GA_3	77.2	118.2	*209.5	30.0	21.6	23.8	73.7	149.1

[a]No stem growth, and flower bud blasting.

tulip bulbs was much stronger than in case of nonprecooled rooted bulbs (Rudnicki *et al.*, 1976). In our experiments we used GA_3 but GA_1, GA_4, GA_9, GA_{12}, GA_{24} and GA_{34} were identified in both non-cold cooled tulip bulb sprouts (Rebers, 1992; Rebers *et al.*, 1994b; 1995). GA_1 was the major gibberellin, while GA_1, GA_9 and GA_{34} were present in lower amounts. Rebers *et al.* (1995) suggested that in tulip, gibberellins are not the cold-induced trigger but are involved in the stalk elongation response, as was shown by the inhibition of this elongation by the GA biosynthesis inhibitors, ancymidol and paclobutrazol, and the reversal of this effect by applied GA (Shoub and De Hertogh, 1974; Saniewski, 1989; Rebers *et al.*, 1994a). Rebers *et al.* (1995) showed the higher level of GA_1 and its inactivation product GA_{34} only in growing floral stalks of cooled bulbs, and the absence of a significant quantitative accumulation of GA_1 and suggest that GA_4 is probably involved in the floral stalk elongation of tulip.

It is possible that excision of all roots in nonprecooled tulip bulbs decreases the level of abscisic acid (ABA) and/or exogenous gibberellic acid is more effective in comparison to intact nonprecooled rooted bulbs. The presence of abscisic acid in tulips is well documented (Aung and De Hertogh, 1979; Singh *et al.*, 1979; Syrtanova *et al.*, 1975; Terry *et al.*, 1982) and low temperature treatment decreases the amount of ABA (Syrtanova *et al.*, 1973; Rakhimbayev *et al.*, 1978; Aung and De Hertogh, 1979). Aung and De Hertogh (1979) suggested that probable sites of abscisic acid biosynthesis in tulip bulb are the developing bulblets, basal plate and roots, since the ABA level is high in these organs and low in the scales and shoot. The inhibitory effect of ABA on pistil and stem growth in tulips was also well documented (Saniewski *et al.*, 1990). It is also possible to consider that after excision of roots in nonprecooled tulip bulbs better penetration and uptake of gibberellin take place and they are more effective in stimulation of shoot growth in

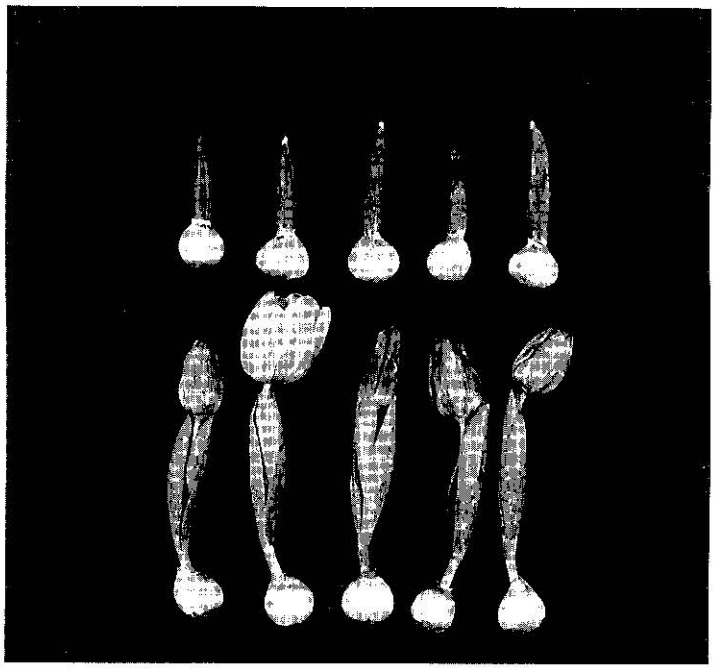

Fig. 2. Effect of leaf apex IAA 0.1% treatment on the shoot growth and flowering of nonprecooled derooted bulbs cv. Gudoshnik kept in water and GA_3 200 mgl^{-1}. Upper row; bulbs kept in water, lower row; bulbs kept in GA_3. From left to right; untreated, lanolin on leaf apex, IAA on leaf apex, lanolin after removal of 2 mm leaf apex, IAA after removal of 2 mm leaf apex. Treatments applied on November 19, photographed on December 21.

comparison to nonprecooled rooted bulbs. Our results indicate that gibberellin can substitute for the cold requirement of tulip bulbs.

ACKNOWLEDGMENTS

The stay of Marian Saniewski at Kyushu University, Fukuoka, Japan was supported by the invitation fellowship program for research in Japan, Japan Society for the Promotion of Science.

REFERENCES

Aung, L. H. and A. A. De Hertogh 1967 The occurrence of gibberellin-like substances in tulip bulbs (*Tulipa* sp.). *Plant Cell Physiol.*, **8**: 201–205

Aung, L. H. and A. A. De Hertogh 1968 Gibberellin-like substances in non-cold and cold treated tulip bulbs (*Tulipa* sp.). *In* "Biochemistry and Physiology of Plant Growth Substances" ed. by F. Wightman and G. Setterfield, Runge Press, Ottawa, pp. 943–956

Aung, L. H. and A. A. De Hertogh 1979 Temperature regulation of growth and endogenous abscisic acid–like content of *Tulipa gesneriana* L. *Plant Physiol.*, **63**: 1111–1116

Aung, L. H. and A. R. Rees 1974 Changes in endogenous gibberellin levels in *Tulipa* bulblets during ontogeny. *J. Exp. Bot.*, **25**: 745–751

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

Cocozza Talia, M. and P. Stellacci 1979 A research on the effect of gibberellin upon tulip flowering. *Acta Hortic.*, **91**: 167–172

De Hertogh, A. 1974 Principles for forcing tulips, hyacinths, daffodils, Easter lilies and Dutch irises. *Scientia Hortic.*, **2**: 313–355

De Hertogh, A. A., L. H. Aung, A. E. Einert and G. L. Staby 1971 Aspects of extractable gibberellin–like substances from *Tulipa gesneriana* L. *Acta Hortic.*, **23**: 90–95

Hanks, G. R. 1984 Factors affecting the response of tulips to gibberellins. *Scientia Hortic.*, **23**: 379–390

Hanks, G. R. 1985 The response of 9 °C–tulips to gibberellins. *Scientia Hortic.*, **27**: 153–161

Hanks, G. R. and A. R. Rees 1980 Growth substances of tulip: the activity of gibberellin–like substances in field–grown tulips from planting until flowering. *Z. Pflanzenphysiol.*, **98**: 213 223

Jones, S. K. and G. R. Hanks 1984 Treatment of tulips with gibberellic acid by vacuum infiltration. *J. Hort. Sci.*, **59**: 241–252

Kawa, L. and M. Saniewski 1986 The effect of gibberellic acid and abscisic acid on tulip pistil growth in vitro. *Acta Hortic.*, **177**: 129–133

Okubo, H. and S. Uemoto 1985 Changes in endogenous gibberellin and auxin activities during first internode elongation in tulip flower stalk. *Plant Cell Physiol.*, **26**: 709–719

Okubo, H. and S. Uemoto 1986 Changes in endogenous gibberellin during internode elongation of tulip flower stalk as affected by light conditions. *J. Japan. Soc. Hort. Sci.*, **55**: 215–220

Okubo, H., S. Shiraishi and S. Uemoto 1986 Factors controlling elongation of the last internode in tulip flower stalk. *J. Japan. Soc. Hort. Sci.*, **55**: 320–325

Rakhimbayev, I. R., G. A. Syrtanova and V. F. Solomina 1978 The effect of cold treatment on the level of biological activity of endogenous growth regulators of tulip bulbs. *Plant Physiol (Russ.).*, **25**: 249–253

Rebers, M. 1992 The role of gibberellins in the cold requirement of tulip. *Acta Hortic.*, **325**: 253–258

Rebers, M., G. Romeijn, E. Knegt and L. H. W. van der Plas 1994a Effects of exogenous gibberellins and paclobutrazol on floral stalk growth of tulip sprouts isolated from cooled and non–cooled tulip bulbs. *Physiol. Plant.*, **92**: 661–667

Rebers, M., E. Vermeer, E. Knegt, C. J. Shelton and L. H. W. van der Plas 1994b Gibberellins in tulip bulb sprouts during storage. *Phytochemistry*, **36**: 269–272

Rebers, M., E. Vermeer, E. Knegt, C. J. Shelton and L. H. W. van der Plas 1995 Gibberellins level and cold–induced floral stalk elongation in tulip. *Physiol Plant.*, **94**: 687–691

Rudnicki, R. M., J. Nowak and M. Saniewski 1976 The effect of gibberellic acid on sprouting and flowering of some tulip cultivars. *Scientia Hortic.*, **4**: 387–397

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

Saniewski, M. and W. J. De Munk 1981 Hormonal control of shoot elongation in tulips. *Scientia Hortic.*, **15**: 363–372

Saniewski, M., L. Kawa and E. Wegrzynowicz 1990 The effect of abscisic acid on pistil and stem growth in tulips. *Prace. Inst. Sad. i Kwiac. Seria B*, **15**: 95–104

Shoub, J. and A. A. De Hertogh 1974 Effects of ancymidol and gibberellins A_5 and A_{4+7} on *Tulipa gesneriana* L. cv. Paul Richter during development in the greenhouse. *Scientia Hortic.*, **2**: 55–67

Singh, B. N., E. Galston, W. Dashek and D. C. Walton 1979 Abscisic acid levels and metabolism in the leaf epidermal tissue of *Tulipa gesneriana* L. and *Commelina communis* L. *Planta*, **146**: 1435–1438

Syrtanova, G. A., I. R. Rakhimbayev and W. I. Kefeli 1975 Native growth inhibitor of tulip. *Plant Physiol (Russ.).*, **22**: 165–169

Syrtanova, G. A., R. Turetzkaja and I. R. Rakhimbayev 1973 Natural auxins and inhibitors in dormant and growing tulip bulbs. *Plant Physiol (Russ.)*., **20**: 1133–1136

Terry, P. H., L. H. Aung and A. A. De Hertogh 1982 Identification of abscisic acid in *Tulipa gesneriana* L. by gas–liquid chromatography and mass spectroscopy. *Plant Physiol.*, **70**: 1574–1576

Van Bragt, J. 1971 Endogenous gibberellin levels and floral stalk elongation in tulip cv. Apeldoorn. *Landbouwwetenschappen Gent*, **36**: 1301–1307

Van Bragt, J. and K. J. van Ast 1976 Substitution of the cold requirement of tulip cv. Apeldoorn by GA_3. *Scientia Hortic.*, **4**: 117–122

Van Bragt, J. and F. A. Zijlstra 1971 Effects of gibberellins on flowering of tulip cv. Apeldoorn. *Z. Pflanzenphysiol.*, **64**: 139–144