

Low temperature sensing in tulip (*Tulipa gesneriana* L.) is mediated through an increased response to auxin

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Received 5 May 1999; Accepted 30 September 1999

Abstract

Tulip (*Tulipa gesneriana* L.) is a bulbous plant species that requires a period of low temperature for proper growth and flowering. The mechanism of sensing the low temperature period is unknown. The study presented in this paper shows that the essential developmental change in tulip bulbs during cold treatment is an increase in sensitivity to the phytohormone auxin. This is demonstrated using a model system consisting of isolated internodes grown on tissue culture medium containing different combinations of the phytohormones auxin and gibberellin. Using mathematical modelling, equations taken from the field of enzyme kinetics were fitted through the data. By doing so it became apparent that longer periods of low temperature resulted in an increased maximum response at a lower auxin concentration. Besides the cold treatment, gibberellin also enhances the response to auxin in the internodes in this *in vitro* system. A working model describing the relationship between the cold requirement, gibberellin action and auxin sensitivity is put forward. Possible analogies with other cold-requiring processes such as vernalization and stratification, and the interaction of auxin and gibberellin in the stalk elongation process in other plant species are discussed.

Key words: Tulip, cold treatment, auxin, gibberellin, mathematical model.

Introduction

A period of low temperature is an essential part of the life cycle of many bulbous plant species. In tulip bulbs (*Tulipa gesneriana* L.) flower development is induced at higher temperatures (20–25 °C), but subsequent elongation of the flower stalk and proper flowering is dependent on an extended period of low temperature (< 10 °C) (De Hertogh and Le Nard, 1993). This dependence of flowering on low temperatures is a well-known phenomenon and the involvement of gibberellins in this cold requirement has been implicated for many cold-requiring plant species such as winter canola (*Brassica napus* cv. Crystal; Zanewich and Rood, 1995), chicory (*Cichorium intybus* L.; Joseph *et al.*, 1983), winter wheat (*Triticum aestivum* L.; Reda *et al.*, 1978), *Thlaspi arvense* L. (Hazebroek *et al.*, 1993), radish (*Raphanus sativus* L.; Michniewicz *et al.*, 1981; Suge, 1970), and also tulip (De Hertogh and Le Nard, 1993). In tulip bulbs, GA application can partly substitute for the required cold treatment (Van Bracht and Zijlstra, 1971; Hanks, 1982; Saniewski and De Munk, 1981). Inhibition of GA biosynthesis greatly reduces stem elongation, both in whole flowering tulip bulbs (Shoub and De Hertogh, 1974; Saniewski, 1989) as well as in isolated sprouts grown *in vitro* (Rebers *et al.*, 1994). This inhibition is reversed by the application of various gibberellins, but no significant increase in endogenous gibberellins is found during cold treatment compared to untreated bulbs (Rebers *et al.*, 1995).

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Abbreviations: DMSO, dimethyl sulphoxide; STS, silver thiosulphate.

Auxins are well-known factors involved in stem elongation in several plant species; a quantitative relationship exists between endogenous indole-3-acetic acid (IAA) levels and growth rate in different genetic lines of garden pea (*Pisum sativum*, Law and Davies, 1990; McKay *et al.*, 1994), in bean stem (*Phaseolus vulgaris* L.; Bialek *et al.*, 1983) and in lupin hypocotyls (*Lupinus albus* L.; Ortuño *et al.*, 1990). Other studies show that exogenously applied IAA promotes stem elongation in light-grown garden pea seedlings (Yang *et al.*, 1993), in hypocotyls of sunflower and marrow (*Helianthus annuus* and *Cucurbita pepo*; Tamini and Firn, 1985) and watermelon (*Citrullus vulgaris*; Carington and Esnard, 1988). However, in the latter two studies, the auxin-induced elongation is short-lived and applied auxin even becomes inhibitory. In tulips, auxin is also necessary for stalk elongation. Removal of the flower bud and leaves, both major auxin sources, before the rapid elongation of the floral stalk reduces floral stalk elongation considerably, whereas application of IAA reverses this effect (Saniewski and de Munk, 1981; Okubo and Uemoto, 1985; Saniewski, 1989). The auxin-induced floral stalk elongation is inhibited by application of paclobutrazol, a GA biosynthesis inhibitor (Saniewski, 1989), suggesting that GA biosynthesis is also necessary for auxin-induced floral stalk elongation. Therefore, interaction of gibberellins and auxins is a prerequisite for stalk elongation in tulips (a view strengthened by the work of Okubo and Uemoto, 1985; Okubo *et al.*, 1986; Saniewski, 1989). Also in other species this interaction seems to be important for stem elongation, for example, cucumber (*Cucumis sativus* L.) hypocotyls (Katsumi and Kazama, 1978), cowpea (*Vigna unguiculata* L.; Okamoto *et al.*, 1995) and garden pea (Yang *et al.*, 1996).

In the work reported here the mechanism of low temperature sensing is addressed and an attempt is made to clarify the role and possible interaction of both auxin and gibberellin in the regulation of cold-induced stalk elongation in tulip. For this a simple *in vitro* system of tulip bottom internodes placed on tissue culture medium supplemented with various concentrations of the two hormones was used (a system adapted from Gabryszewska and Saniewski, 1983). The internodes were isolated from tulip bulbs that have been cooled for different periods of time to study the effect of a low temperature treatment on the ability to respond to the different hormones. The obtained dose–response curves are described in terms of a mathematical equation, adopted from the field of enzyme kinetics (as suggested by Weyers *et al.*, 1987). The equation is fitted to the data using regression to obtain a working model for the regulation of stalk elongation by gibberellin, auxin and low temperature treatment. Possible analogies with stalk elongation in other plant species are discussed.

Materials and methods

Plant material

In vitro experiments were performed with stalks from field-grown bulbs (*Tulipa gesneriana* L. cv. Apeldoorn) harvested in July 1995 (experiment A) and July 1994 (experiment B). Bulbs (circumference 11–12 cm for experiment A and 12–13 cm for experiment B) were stored in dark ventilated rooms at 20 °C.

Internodes were isolated from tulip bulbs by isolating whole sprouts. These were surface sterilized (ethanol rinse for 30 s followed by rinsing in 1% NaClO for 30 min and three consecutive washes with sterilized demineralized water). In a sterile flow cabinet bottom internodes were then isolated from the sprouts and placed inverted (topside in the medium) in glass tubes with 10 ml of solid medium. All internodes were incubated in the dark at 24 °C.

For experiment A, the bulbs were transferred on September 22 1995 to dark ventilated rooms at 17 °C. After 12 weeks the cold treatment began by transferring the bulbs to dark ventilated rooms at 5 °C. Internodes were isolated from the bulbs and placed on medium as described above, after 0 (22 December 1995, start of the treatment), 4 (19 January 1996), 8 (16 February 1996) or 12 weeks (15 March 1996) of cold treatment.

For experiment B, the bulbs were transferred on September 23 1994 to dark ventilated rooms at 5 °C for the cold treatment, or 17 °C for the non-cooled controls. After 12 weeks internodes were isolated from the bulbs and placed on medium as described above.

Growth medium and auxin and gibberellin application

Internodes were incubated on standard Murashige and Skoog (Murashige and Skoog, 1962) medium (Duchefa, Haarlem, the Netherlands), with 30 g l⁻¹ sucrose added and 0.8% agar (Duchefa). The medium was autoclaved for 20 min. To prevent possible ethylene effects, filter-sterilized (0.22 µm disposable filter, Amicon) silver thiosulphate (STS) solution was added to the medium to a final concentration of 50 µM. Indole-3-acetic acid (IAA, Duchefa) was added from a filter-sterilized stock-solution (10 mM). GA₃ (Duchefa.) stock solution was prepared by dissolving it in dimethylsulphoxide (DMSO) to a final concentration of 100 mM. For the lower GA₃ concentrations a 10 times diluted stock was used. DMSO concentration in the media was, if necessary, corrected by adding DMSO to a final concentration of 0.1% (v/v). STS, IAA and GA₃ were added after autoclaving.

In experiment A internodes from 0, 4, 8, and 12 weeks cooled bulbs were grown on media containing a range of eight concentrations of IAA (0, 0.1, 0.3, 1, 3, 10, 20, and 30 µM) combined with two concentrations of GA₃ (0 and 100 µM). For every hormone combination 10 internodes were used. In experiment B, internodes from both cooled and non-cooled bulbs were grown on media containing IAA as above combined with a range of six concentrations GA₃ (0, 0.3, 1, 3, 30, and 100 µM). Per combination 10 internodes were used, both for cooled and uncooled bulbs.

Data analysis

The elongation of the internodes was monitored for 4 weeks. Sometimes internodes did not show a growth response, and later started to show a callus-like growth, especially at higher IAA concentrations. These internodes and any infected ones were discarded. On average 2.7 internodes per hormone combination were discarded, resulting in average 7.3 usable

samples per hormone combination. The lengths of internodes for every IAA-GA₃ combination were averaged for every time point and a logistic growth-curve was fitted through them from which the maximum elongation rate (mm d⁻¹) during these 4 weeks was determined. If a logistic growth curve could not be fitted (at low IAA concentrations and in zero or short cold treatments) the maximum elongation rate was determined as the maximum rate of change between two data points. The maximum rate is normally reached within the first week of growth. The maximum elongation rate, i.e. the slope at the steepest point in the growth curve, throughout this study is referred to as the response of the internodes to a combination of hormones (as suggested by Weyers *et al.*, 1987).

Results

Adapting an in vitro system for studying hormonal regulation of low temperature-dependent stalk elongation

The tulip flower stalk is only able to elongate rapidly after a minimally required cold treatment (De Hertog and Le Nard, 1993). In field-grown bulbs, the largest relative change in internode elongation and elongation rate after different periods of low temperature treatment occurs in the bottom internode, which is the first to begin elongating after planting (data not shown).

To investigate this further, bottom internodes were placed inverted on solid tissue culture medium supplemented with auxin (1 μM), gibberellic acid (GA₃, 10 μM) or both. At these hormone concentrations the elongation rate of the bottom internode is mainly determined by auxin, with an enhancing effect by low temperature treatment of the bulbs and the presence of gibberellic acid (Fig. 1). Both latter factors are themselves not sufficient to induce a significant increase in elongation compared to the control. The endogenous hormone level in the bottom internodes is not sufficient for induction of a rapid elongation rate.

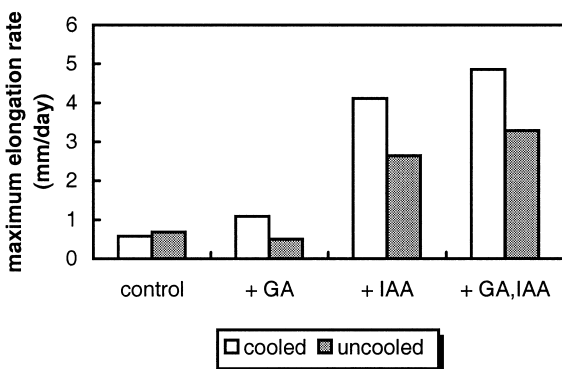


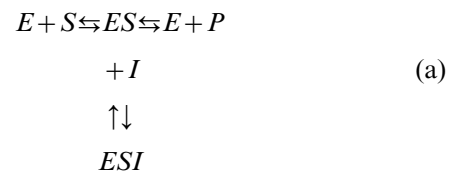
Fig. 1. Effect of cooling and plant hormones on the elongation of the bottom internode. Tulip bottom internodes of bulbs stored for 12 weeks at 5 °C or 17 °C, were incubated on tissue culture media containing no plant hormones, 1 μM IAA, 10 μM GA₃ or both. The length of the internode was recorded over 4 weeks. The maximum elongation rate over this time period is shown. Mean se is 0.3 (mm d⁻¹).

The *in vitro* system was used for a detailed evaluation of the hormonal regulation of low temperature-dependent internode elongation. Experiment B shows that both the cold treatment and the addition of GA₃ to the medium enhance the elongation response of the internodes to auxin and shift the maximum response to lower auxin concentrations (Fig. 2C and D versus A and B, and A and C versus B and D, respectively). The combination of cold storage of the bulbs and addition of GA₃ to the medium has the largest effect on the elongation.

The auxin response is analysed as the maximum observed elongation rate over the time-course of the elongation curve (as suggested by Weyers *et al.*, 1987).

Auxin dose-response curves fit equations for uncompetitive inhibition of enzyme kinetics

Interaction between a hormone and its receptor can be considered as substrate enzyme interactions (Weyers *et al.*, 1987), as long as the same assumptions and simplifications are applied as in the Michaelis-Menten model for enzyme kinetics. At higher IAA concentrations, elongation was inhibited and this inhibition was independent of ethylene (data not shown). Therefore, equations describing different kinds of inhibition were fitted to the dose-response curves. The equations are derived from the Michaelis-Menten model for saturated enzyme kinetics. The equation that fits best is an adapted equation describing uncompetitive inhibition (Segel, 1975). The reaction scheme is as follows:



where *E* is free enzyme, *S* is substrate, *ES* is the enzyme-substrate complex, and *P* is the reaction product. The corresponding equation for uncompetitive inhibition is given by:

$$v = \frac{V_m s}{s + K_m + si/K_i}
 \tag{1}$$

where *v* is the steady-state reaction rate, *s* is substrate concentration, *i* is inhibitor concentration, *K_m* is the affinity constant, a measure for the affinity of the enzyme for its substrate, *V_m* is the maximum velocity rate, and *K_i* is an affinity constant, a measure for the affinity of the enzyme for the inhibitor.

Replacing the terms substrate and enzyme with hormone and receptor, respectively, and replacing the term

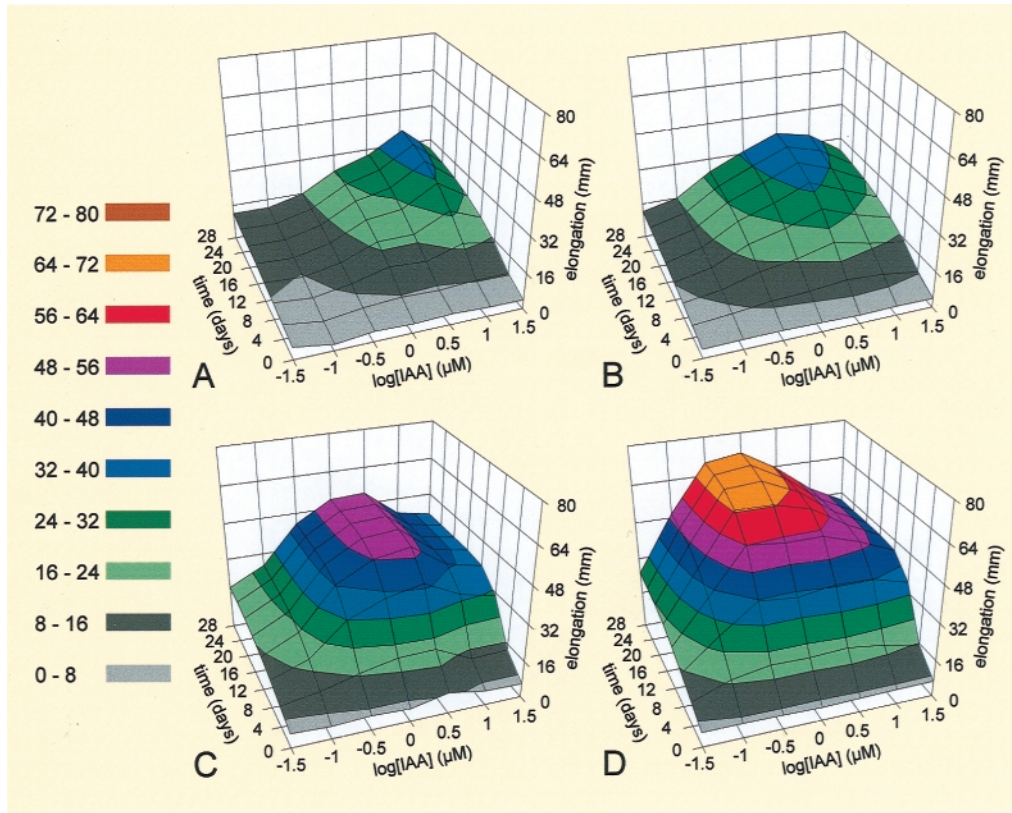
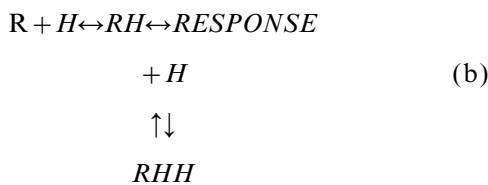


Fig. 2. Effect of cooling and plant hormones on the growth curves of bottom internodes. Tulip bottom internodes of bulbs stored for 12 weeks at 5°C or 17°C, were grown on tissue culture media containing a range of eight IAA concentrations. The length of the internodes was recorded over time. (A) 17°C, no GA₃ in the medium. (B) 17°C, 100 μM GA₃ in the medium. (C) 5°C, no GA₃ in the medium. (D) 5°C, 100 μM GA₃ in the medium. The elongation curves shown are fitted elongation curves for data obtained from 7.3 average separate internodes per IAA concentration.

inhibitor by hormone, results in the following scheme:



where *R* is the receptor, *H* is the hormone, *RH* is the hormone-receptor complex, resulting in a *RESPONSE*. Likewise in the derived equation the terms *s* and *i* are both substituted by *h*, the hormone concentration, resulting in:

$$v = O + \frac{V_m h}{h + K_m + h^2/K_i} \quad (2)$$

The terms *v*, *V_m*, *K_m*, and *K_i* have the same meaning as in equation 1, although the affinity constants are now describing affinity of the receptor for its ligand (*K_m*) or for its ligand as an inhibitor (*K_i*). *O* is an offset constant describing the very low basic elongation rate without any auxin in the medium, possibly due to endogenous auxin. Equation 2 was used for fitting the dose–response curves.

Cold treatment gradually enhances auxin response

In experiment A the elongation curves of the bottom internodes at different auxin concentrations were recorded and the response to auxin was determined as described above after 0, 4, 8 or 12 weeks of cold storage. Auxin dose–response curves were fitted using the equation described previously. Increasing the length of the cold storage has a significant influence on the response of the internodes to auxin (Fig. 3A). The maximum response level of the internodes increases with increasing length of the cold treatment. This is further referred to as the responsiveness of the internodes to IAA. The corresponding IAA concentration at which the maximum response is reached (*IAA_{max}*) is further referred to as the sensitivity. Thus, a lower *IAA_{max}* means a higher sensitivity. The maximum response and corresponding *IAA_{max}* can be calculated using the estimated parameters of the fitted equations (Table 1). The cold treatment enhances the responsiveness dependent on its duration, leading to a 3-fold increase after 12 weeks of treatment. The cold treatment also increases the sensitivity of the internodes, although this effect is more pronounced during the first 8 weeks of the treatment.

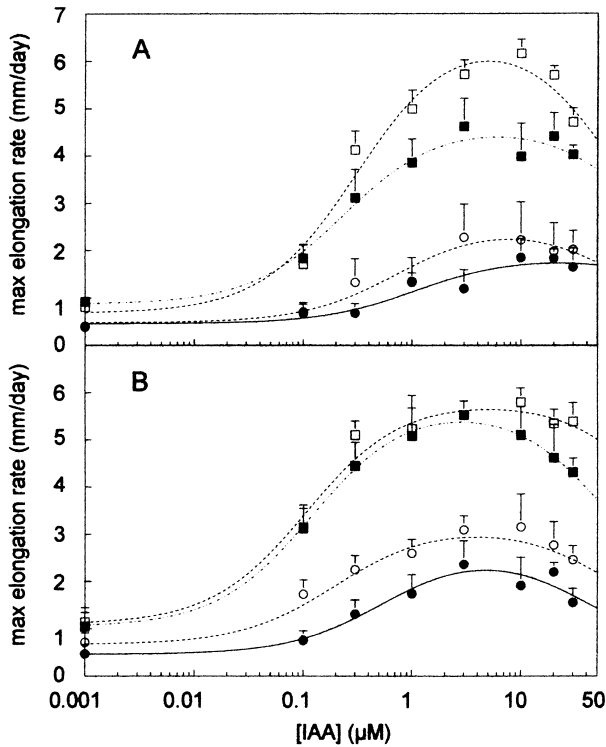


Fig. 3. Effect of the length of the cold treatment on the auxin dose-response curves. Tulip bottom internodes of bulbs stored at 5 °C for different periods of time were incubated on tissue culture media containing a range of eight IAA concentrations. The length of the internodes was recorded, and the maximum elongation rate was determined. This maximum elongation rate is the response of the internode to auxin. Dose-response curves were fitted for internodes grown on medium without GA₃ (A) and with 100 μM GA₃ (B). Curves are fitted as described in Materials and methods. The data points and error bars are shown. 0 weeks at 5 °C (—●—), 4 weeks at 5 °C (---○---), 8 weeks at 5 °C (—■—), 12 weeks at 5 °C (---□---). Per data point an average of 7.3 internodes was used, so per curve an average of 58.4 internodes was used.

GA₃ in the medium enhances the response to auxin independent of low temperature treatment

The addition of GA₃ enhances both the responsiveness and sensitivity of the internodes to auxin (Fig. 3B; Table 1). However, after 12 weeks of cold treatment the

effect of GA₃ in the medium on both parameters is minimal, although a general broadening of the dose-response curve can be observed (Fig. 3B versus 3A).

To determine the effect of different GA₃ concentrations in the medium on the auxin response of bottom internodes, the elongation curves at different auxin and gibberellin concentrations were recorded and analysed as described above. Increasing amounts of GA₃ result in a gradual shift of the dose-response curves. Both the responsiveness and the sensitivity are increased, for both cooled or non-cooled tulip bulbs (Table 2). The addition of >30 μM of GA₃ to the medium does not lead to an additional effect, either on the maximum response or on the IAA_{max}. These results confirm those of a similar experiment performed in the season 1993/1994 (data not shown).

Discussion

Rapid floral stalk elongation in tulips only occurs with bulbs that have received a low temperature treatment. This study clearly shows that the effect of the cold treatment is on the response to auxin, which is the main hormonal factor involved in the induction of the elongation *in vitro*. Upon cooling, the internodes show a higher responsiveness and sensitivity to auxin (Table 1; Figs 2, 3). This change in the response of the bottom internodes to auxin gradually increases with the length of the cold treatment. However, gibberellin without auxin is not able to cause a significant elongation, and thus auxin is the main factor determining the elongation rate (Figs 2, 3). At higher auxin concentrations in the medium (IAA > 10 μM) the elongation rates dropped (Fig. 3). Ethylene was eliminated as an interacting factor because no significant ethylene production could be detected during elongation and the addition to the medium of silver thiosulphate (STS), an inhibitor of ethylene action, had no effect on the reduction of elongation rates at higher auxin concentrations (data not shown).

The role of gibberellic acid

Adding gibberellin to the growth medium has an effect on the auxin response that is independent of, but very

Table 1. Effect of the duration of the cold treatment of tulip bulbs on the *in vitro* elongation of dark-grown bottom internodes

Tulip bottom internodes that have been given different periods of cold treatment were grown *in vitro* in the dark on media with a range of IAA concentrations. IAA dose-response curves were fitted for each treatment. The maximum response and corresponding IAA concentration were determined from the fitted dose-response curves. Parameters correlate to the data in Fig. 4A (without GA₃) and 4B (with 100 μM GA₃). Standard fit errors are in parentheses. For the construction of each curve an average of 58.4 internodes was used.

Weeks of cold treatment	0 μM GA ₃		100 μM GA ₃	
	Maximum response (mm d ⁻¹)	IAA concentration (μM)	Maximum response (mm d ⁻¹)	IAA concentration (μM)
0	1.74 (0.13)	22.0 (67.2)	2.25 (0.18)	4.70 (1.08)
4	2.23 (0.17)	7.60 (2.63)	3.05 (0.12)	3.98 (0.76)
8	4.41 (0.18)	5.98 (1.84)	5.41 (0.07)	2.85 (0.14)
12	6.04 (0.32)	4.99 (1.03)	5.68 (0.19)	5.01 (1.92)

Table 2. Effect of GA₃ on the *in vitro* elongation of dark-grown tulip bottom internodes

Tulip bottom internodes, isolated from cold-treated and untreated tulip bulbs, were grown *in vitro* in the dark on media with a range of IAA concentrations and a range of GA₃ concentrations. IAA dose–response curves were fitted for each GA₃ concentration. The maximum response and corresponding IAA concentration were determined from the fitted dose–response curves. Standard fit errors are in parentheses; n.d. means that the standard fit error could not be determined because the fit did not converge. For the construction of each curve an average of 58.4 internodes was used.

GA ₃ concentration (μM)	12 weeks at 5 °C		12 weeks at 17 °C	
	Maximum response (mm d ⁻¹)	IAA concentration (μM)	Maximum response (mm d ⁻¹)	IAA concentration (μM)
0	3.84(0.25)	5.69(4.32)	2.54(n.d.)	7.03 (n.d.)
0.3	4.53 (0.54)	2.98(1.66)	2.96(0.26)	3.91(0.97)
1.0	5.21(0.32)	2.30(0.43)	2.89(0.28)	3.83(1.13)
3.0	4.99(0.43)	1.34(0.51)	3.49(0.34)	3.81(0.91)
30	5.62(0.36)	1.30(0.38)	3.74(0.14)	2.73(0.34)
100	5.83(0.39)	1.29(0.33)	3.49(0.23)	2.32(0.48)

similar to the effect of a cold treatment. A higher gibberellin concentration results in a higher responsiveness and higher sensitivity to auxin (Table 2). At GA₃ concentrations higher than 30 μM a plateau in the effect of GA₃ on the dose–response curves is observed. That gibberellin is a necessary component in the elongation process, is illustrated *in vitro* by the effect of paclobutrazol application on the elongation of complete sprouts (Rebers *et al.*, 1994). It reduces stem elongation severely, and its effect is reversible by gibberellin application. This need for gibberellin during the elongation phase is also present *in vivo* as the addition of gibberellin biosynthesis inhibitors after planting inhibits elongation, with its main effect in the bottom internode (Shoub and De Hertogh, 1974; Saniewski, 1989; Suh *et al.*, 1992). The site for GA biosynthesis appears to be the growing root system as removal of the roots inhibits stalk elongation, which could be reversed by the addition of GA₃ (Kawa-Miszczak *et al.*, 1992).

The maximum response levels and optimum auxin concentration in Tables 1 and 2 cannot be compared in an absolute way because the experiments were performed in two different seasons. The main difference between the data in the two tables is the magnitude of the gibberellin effect. The effect of GA₃ on the response levels and optimum auxin concentration in Table 2 is higher (than in Table 1). The cold storage for the experiment resulting in the data of Table 1 ended later in the season than for the experiment resulting in the data of Table 2. Also, *in vivo*, stalk elongation is reported to be more rapid later in the season, suggesting a reduced need for gibberellins at that time (Saniewski, 1989).

It is postulated that a cooling period is needed to increase the sensitivity of the tulip sprout for gibberellins (Hanks, 1982). However, the results in this paper show that gibberellins are not the main factor involved in cold-induced stem elongation, although it is clear that *de novo* synthesis of gibberellic acid is a prerequisite for stalk elongation in tulip bulbs. The hypothesis for this study is

that *in vivo* the auxin response in the internodes of non-cooled bulbs is not sufficient for proper elongation. Only after proper cold treatment and the resulting shift in the auxin response are the internodes able to elongate at a proper rate, but only in the presence of certain amounts of gibberellins. After cold treatment, GA₃ is able to induce an even greater enhancement of the auxin response, but late in the season this effect is less clear. GA₃ alone is not able to induce the change in the auxin response that can be induced by an optimal cold treatment. Thence gibberellins and cold treatment may not operate via the same modi. They affect the auxin response in separate ways, but with a similar result.

The involvement of gibberellins in vernalization and stratification is well known in many plant species (Metzger, 1995; Rock and Quatrano, 1995), but until now little has been reported about a decisive role for auxin sensitivity in low temperature-induced processes. Interaction of auxin and gibberellins as being important for stem elongation is suggested also for other species, as described in the Introduction. In one study in particular on garden pea, the stimulating effect of GA is mainly attributed to an increase in cell number indicating that GA stimulates cell division pea (Yang *et al.*, 1996). It was shown that, in tulip, the elongation of the floral stalk after planting is due to cell elongation only (Gilford and Rees, 1973).

Mathematical modelling

The curve-fitting procedure used in this study is based on suggestions made by Weyers *et al.* (Weyers *et al.*, 1987). They suggest that hormone receptor interaction can be considered as substrate enzyme interaction. This technique has been applied by other authors to describe the effect of ethylene on flower senescence (Woltering *et al.*, 1993), of nitrate on light-induced germination (Hilhorst, 1990) and of abscisic acid on stomatal closure (Weyers and Paterson, 1992). Although several different equations

taken from the field of enzyme kinetics were used for curve fitting, the best results were obtained with the one adapted from the model for uncompetitive inhibition (data not shown). In all dose–response curves the inhibitory effect of IAA at high concentrations is evident. However, it is only possible to speculate on the significance of this for the molecular mechanism of auxin action, although the results exclude the involvement of ethylene. It is suggested that reaction scheme b is best interpreted in such a way that the equation term RH is not considered as the real hormone–receptor complex, but as a general term describing all the steps from the hormone binding to its receptor to the final changes in the cells that cause the elongation. The inhibition by IAA can take place on any of these events in the chain leading from receptor binding, but also on events preceding this binding, such as activities of conjugating enzymes or transport of exogenous hormone.

The most important reason to describe the dose–response curves by mathematical equations is to be able to compare the curves in a quantitative way and thus compare the effects of low temperature and gibberellin on auxin perception. In this study it is not meant to elucidate what the molecular mechanism is of auxin perception itself.

Low temperature sensing by a change in the response to auxin

Figure 4 depicts a hypothetical model for low temperature induction of stalk elongation in tulip. It is shown that auxin is the hormone responsible for the elongation of the bottom internode in tulip, with both cold treatment and gibberellins enhancing the response to auxin. In the model the auxin perception complex must be interpreted as all steps between binding of auxin to its receptor to the actual elongation, and thus includes signal transduction events, primary and later gene induction events

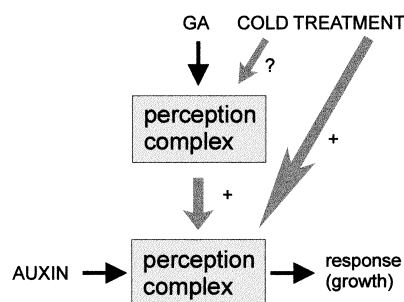


Fig. 4. Working model for the different components involved in the low temperature induced stalk elongation in tulip. Auxin causes an elongation response in the floral stalk through the perception complex consisting of a receptor and subsequent signal transduction pathway. Cold treatment and gibberellic acid have an enhancing effect on the auxin perception complex. The effect of the cold treatment on the gibberellic acid perception complex is not clear and indicated by a question mark. Grey arrows indicate a modulating effect.

resulting in elongation. These data and data from the literature suggest that this change in auxin response is the main factor responsible for the different growth behaviour *in vivo* of cold-treated versus untreated tulip bulbs and thus that low temperature sensing in tulip is mediated by this change in auxin response. It is a clear example of modulating plant development by changing the sensitivity for a plant growth regulator (as presented by Trewavas, 1981, 1982). However, the results show that auxin acts as a real plant hormone, contrary to the view described in these papers. The effective range of the dose–response curves spans only two orders of magnitude and auxin can thus effectively regulate elongation. Internode elongation in tulip is thus a case of combined control (a mechanism proposed by Weyers *et al.*, 1995). The low temperature changes the response to auxin, but the actual elongation only occurs when auxin is supplied (for example by the leaves or flower bud).

In tulip, stalk elongation is dependent on low temperature, but flower formation is not. In some other bulbous species like iris, both the initiation of flower development and stalk elongation are dependent on reduced temperatures (De Munk and Schipper, 1993). This is also true for many vernalizable plant species, and it would be interesting to investigate if increased auxin sensitivity occurs also in these species. Presently, the tulip is being used as a model system for the isolation of genes involved in low temperature regulation and auxin sensitivity. The advantage of this system is the absence of interfering processes such as flower development as is the case in many vernalizable species, especially in crucifers like *Thlaspi arvense* (Hazebroek *et al.*, 1993).

In conclusion, cold treatment and gibberellin positively enhance the auxin response in the bottom internode in tulip, and thus its potential to elongate rapidly. It is hypothesized that *in vivo* rapid elongation only occurs when the sensitivity to auxin is increased sufficiently by a low temperature treatment and that for optimal elongation, interaction of auxin and gibberellins is important.

Acknowledgements

We thank Dr E Gabryszewska and R Luyten for their assistance during the initial part of this work. We also thank Drs EJ Woltering and GJA Rouwendal for critically reading of the manuscript. This work was supported in part by the Life Sciences Foundation (SLW), which is subsidized by the Netherlands Organisation for Scientific Research (NWO).

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