THE ROLE OF GIBBERELLINS IN THE COLD REQUIREMENT OF TULIP

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Abstract

The involvement of gibberellins (GAs) in the regulation of stem elongation and flowering has been implicated in cold requiring plants, including tulip. To investigate their role in tulip, research is directed towards qualitative and quantitative analyses of endogenous GAs and the biological activity and metabolism of applied GAs.

At first, an inventory was made of GAs including the conjugated forms, in sprouts of cooled and uncooled bulbs (*Tulipa gesneriana* L. cv. Apeldoorn). By combined gas chromatography-mass spectrometry (GC-MS) and GC-selected ion monitoring (SIM), GA_4, GA_9, GA_{24}, GA_{34} and four GA-related compounds were detected. Most detected GAs and GA-related compounds were found in the free as well as in the conjugated form and occurred in sprouts of both cold and non-cold treated bulbs.

The biological activity of GA_4 and GA_9, and the not detected but in many plants active GA_1, was tested by applying them in vitro to sprouts of uncooled and cooled bulbs, in the presence or absence of the GA-biosynthesis inhibitor paclobutrazol. Stem elongation and flower quality in this in vitro system were poor, even after an optimum cold treatment of the bulbs. The sensitivity to applied GAs increased during cold treatment and the three GAs were equally effective in stimulating stem elongation. Paclobutrazol had no effect on stem length, but flower quality had increased in paclobutrazol treated compared to untreated sprouts.

Additional index words
GC-MS, *Tulipa gesneriana*, stem elongation, flowering, paclobutrazol

1. Introduction

Tulip bulbs, with terminal buds containing a complete flower, require a period of low temperature for floral stalk elongation and adequate flowering. In these processes the involvement of gibberellins has been implicated (Saniewski, 1989).

Two hypotheses have been put forward to explain their role:

1) During the cooling of the bulbs the amount of free GAs increases (Saniewski, 1989), by activation of the GA biosynthesis (Saniewski, 1989; Metzger 1990), or by hydrolysis of conjugated GAs or prevention of the conjugation of free GAs. It is possible that reversible GA-conjugation is involved in the regulation of biological active hormone concentrations (Sembdner et al., 1991).

2) During the cooling the sensitivity to GAs increases (Hanks, 1982).

An inventory of endogenous GAs is a prerequisite for further study. The introduction of gas chromatography-mass spectrometry provides an advanced method

for both qualitative and quantitative analyses of GAs.

The biological activity of some crucial GAs was tested in combination with the GA-biosynthesis inhibitor paclobutrazol, using isolated sprouts in in vitro assays. Paclobutrazol, applied to whole tulip bulbs either before or after cooling, should inhibit stem growth but allows normal development of leaves and flowers (Saniewski, 1989; Suh et al., 1992).

2. Material and methods

2.1 Material

Field grown tulip bulbs (*Tulipa gesneriana* L. cv. Apeldoorn) were harvested in July and stored at 20°C until start of treatment in October. Then the cold treatment (5°C) was started for different time periods: 0, 6 and 12 weeks. Bulbs kept at 17°C served as controls. The reaction of bulbs to transfer to higher temperatures after cooling, was studied with bulbs stored at 17°C for one week after 12 weeks at 5°C. After the temperature treatments the sprouts were isolated from the bulbs. For the GA analyses, the material was stored at -75°C until extraction. For the application of GAs in vitro, sprouts were processed directly.

2.2 Method

2.2.1 Qualitative GA analyses

The low levels of GAs in tulip sprouts require an extended purification prior to analysis by GC-MS. Sprout material (50 g FW) was extracted with methanol in the presence of small amounts of the tritiated GA standards [^{3}H]GA_1, [^{3}H]GA_4 and [^{3}H]GA_9. The extracts were purified by solvent partitioning, anion exchange chromatography and reversed phase gradient HPLC according to the method of Croker et al (1990), with some adaptations. Conjugated GAs were hydrolyzed enzymatically with a cellulase preparation of *Aspergillus niger* (Sigma) and analysed as free GAs. The effluent from the HPLC was collected in 7 fractions. Putative GA containing fractions were methylated and rerun over reversed phase HPLC using the same gradient. Putative methylated-GA containing fractions were collected, silylated and separated by GC over a capillary apolar column CP-SIL 8CB. Gibberellins were detected by mass spectrometry and analysed using full-scan and selected ion monitoring (SIM) with a Hewlett Packard 5970 system.

2.2.2 GA and paclobutrazol application

The isolated sprouts were surface sterilized, washed and inoculated in glass tubes with 1 ml of liquid medium (Murashige and Skoog, 1962) + 8% sucrose and 2 $mg.l^{-1}$ of thiamine HCl, d-biotine, nicotinic acid and pyridoxine. GAs were added in amounts of 0, 10 or 100 μg per sprout, in the presence or absence of 35 μg paclobutrazol (maximum solubility). The sprouts (10 per treatment) were grown at 20°C with a 16h light period. Used medium was replenished with medium without GA or paclobutrazol. Weekly measurements were made until growth had stopped.

Then the sprouts were removed from the tubes and stem lengths were measured.

3. Results and discussion

An inventory was made of GAs including the conjugated forms, in sprouts from bulbs cold treated for different time durations. The presence of GA_4, GA_9 and GA_{24} could be demonstrated, in estimated amounts less than 100 $ng.g^{-1}$ fresh weight. Traces of GA_{34} were indicated by GC-SIM. Additionally four GA-related compounds were detected (a.o. OH-GA_{12}). Most detected GAs and GA-related compounds were found in the free as well as in the conjugated form and occurred in sprouts of both cold and non-cold treated bulbs. The detected GAs belong to two families of GAs with different hydroxylation patterns (figure 1): the non- and the 3-hydroxylated GAs. Remarkably the 13-hydroxylated GAs (e.g. GA_1), present in many plant species, were not detected in any of the extracts.

Current research is directed towards quantitative analyses using deuterated GA_4, GA_9, GA_{24} and GA_{34}, in relation to the cold pretreatment of the bulbs.

The biological activity of GA_1, GA_4 and GA_9, was tested by applying them in vitro to sprouts of cooled and uncooled bulbs. In figure 2 the maximum effects of the GA applications are shown and in figure 3 the stem lengths in relation to bulb treatment and GA are summarized. Stem elongation in these in vitro grown sprouts was rather poor, even after the optimum bulb treatment of 12 weeks at 5°C. The stem lengths of the control sprouts without applied GA hardly reached 50 mm, while in field grown tulips stem lenghts generally yield 400 mm or more.

The sensitivity of the sprouts to GAs increased during the cold treatment of the bulbs (figure 2, 3). In sprouts of 12 weeks cooled bulbs application of GAs yielded considerably larger stem elongation than GA application to sprouts of uncooled bulbs (17°C for 12 weeks). The maximum effects of the three tested GAs were not significantly different. Even GA_1, that has not been detected in any of the sprout extracts, yielded comparable stem elongation. For this reason the possibility of a general, aspecific GA effect should be considered.

The transfer to 17°C for one week after 12 weeks at 5°C, resulted in a decrease in sensitivity to GA_1 and GA_9.

The growth retardant paclobutrazol was used to study the role of GA synthesis in the cold induced stem elongation. The results are summarized in figure 4. Unexpectedly paclobutrazol did not inhibit stem elongation. This may have had several reasons: 1) GA synthesis was not involved in the cold induced stem elongation, 2) GA synthesis did not occur due to inadequate in vitro conditions, or 3) at the site of GA synthesis the concentration of paclobutrazol was too low. Remarkable was the positive effect of paclobutrazol on the flower quality of sprouts from cooled bulbs. Out of 60 sprouts treated with GA and paclobutrazol, 24 yielded well developed flowers, while without paclobutrazol only 2 out of 60 yielded well developed flowers. These results showed that paclobutrazol had been transported into the sprouts. This effect on flowering can not be explained by inhibition of the GA synthesis only. In several plants paclobutrazol has been shown to increase cytokinin contents (Izumi & Oshio, 1991), that possibly accounts for these results.

Due to the poor stem elongation in this in vitro system, it did not become clear if GA application could replace the cold treatment of the bulbs. The role of GA-

synthesis in the cold requirement of tulip bulbs is not elucidated yet, but the increase in sensitivity to GAs during cold treatment is clearly demonstrated.

In future research the effect of paclobutrazol will be compared with BX-112, an inhibitor of a.o. the hydroxylation of $GA_9 \rightarrow GA_4$ (Rademacher, 1991).

4. Acknowledgements

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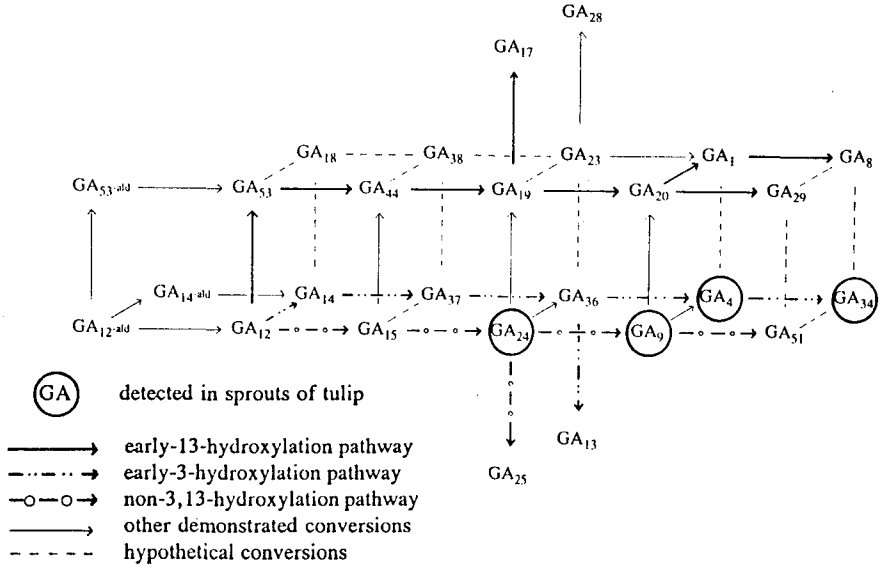

Figure 1 - Metabolic grid of GAs in higher plants showing known sequences and the GAs detected in sprouts of tulip bulbs. Adapted from Sponsel (1987).

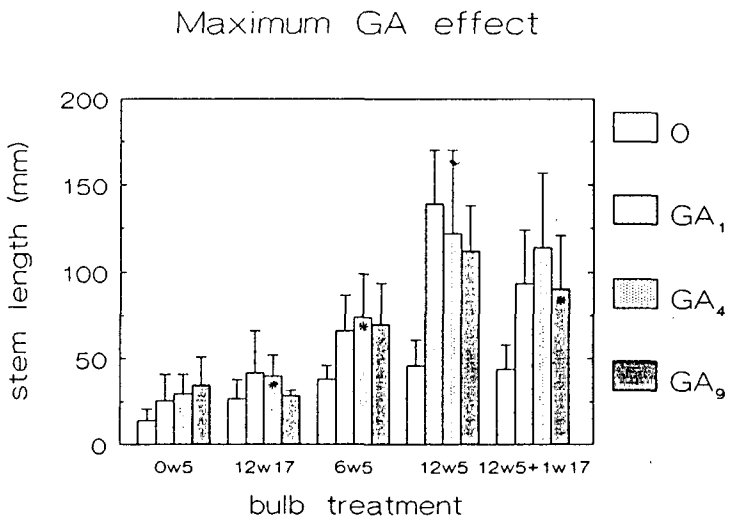

Figure 2 - Maximum effects of GA application in relation to bulb treatment. Sprouts were inoculated with 1 ml medium containing 0, 10 or 100 μg GA 1, 4 or 9. Stem lenghts were measured after respectively 19, 8, 14, 8 and 8 weeks growth in vitro. The amounts yielding the largest responses are shown; this was generally 100 μg and sometimes 10 μg (marked with asterisks).

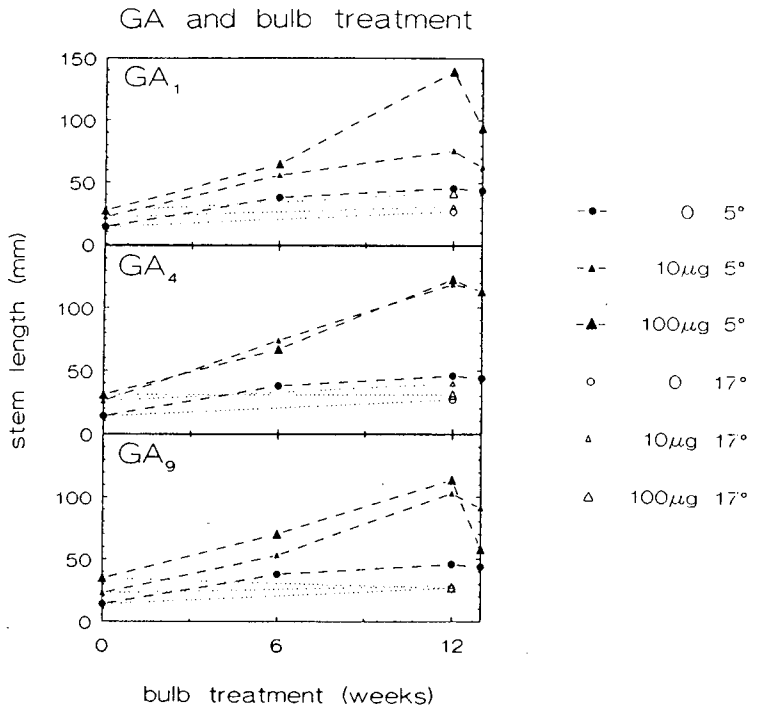

Figure 3 - Effects of GA application in relation to bulb treatment. Sprouts were inoculated with 1 ml medium containing 0, 10 or 100 µg GA 1, 4 or 9. Stem lenghts were measured after respectively 19, 14, 8, 8 and 8 weeks of growth in vitro.

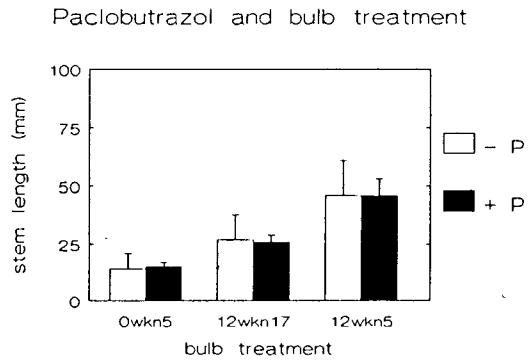

Figure 4 - Effect of paclobutrazol and bulb treatment on stem elongation of sprouts from cooled (12 weeks at 5°C) and uncooled (0 weeks at 5°C and 12 weeks at 17°C) bulbs. Sprouts were inoculated with 1 ml medium in the presence or absence of 35 µg paclobutrazol. Stem lenghts were measured after 19, 8 and 8 weeks growth in vitro respectively.