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ARTICLE

Phytohormones dynamics during flowering initiation in carrots

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ABSTRACT This study was aimed on investigation of phytohormones dynamics in carrot (*Daucus carota* L.) during different flowering initiation stages. Different levels of development were needed for photo- and thermo-induction; tipically 5 and 9 leaves in rosette for the first and second flowering induction stages, respectively. The process of carrot flowering initiation and morphogenesis was studied in a phytotron facility. High-performance liquid chromatography was used for separation of phytohormones. The best rate of development was found during exposure to florally inductive effect with low temperature either a short day or long day photoperiod in carrots with 9 leaves in rosette. Temperature influence had higher effect on phytohormones biosynthesis than differences in photoperiod. Antagonistic as well as stimulatory steps were involved during hormonal action and the balance of these determined the final effect of even a single hormone. The ratio of investigated phytohormones had substantial influence on flowering initiation processes. An increase of gibberellic acid content in evocation stage II and decrease in flower initiation stage determined faster stem elongation and bud formation. Decrease of abscisic acid and increase of gibberellic acid level in evocation stage II could be related with fast flowering induction processes. The highest indol-3 acetic acid concentration in evocation stage II induced the formation of inflorescence axis structures.
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KEY WORDS

carrot
evocation
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There exist several hypotheses, concepts and theories for explaining the mechanism of plant transition to the generative development (Chailakhyan 1988; Jordan 1993). In most of these theories, various aspects of the hormonal regulation of flowering were analyzed. It has been proposed that phytohormones are involved in the metabolism pathway, which occurs after light perception and transduction of the initial signal into a physiological effect (Kraepiel and Miginiac 1997). The carrot plant flowers only after vernalization, *i.e.*, low temperatures are needed for flower initiation (Dias-Tagliacozzo and Válio 1994). Reproductive success of plant largely depends on the correct timing of floral induction. This is the reason why the initiation of flowering is highly regulated by environmental cues exhibiting regular seasonal changes, such as photoperiod and temperature, and by the developmental stage of the plants (Bernier et al. 1993). The end of the juvenile period is linked to plant's utter preparation for photo- and thermo induction processes. In carrot ontogenesis this moment corresponds with the beginning of carrot root thickening. At that moment plants form 8 assimilating leaves. Photo- and thermo induction mechanisms are not interrelated, they can proceed at different time and stipulate different effects (Duchovskis et al. 2003). According to Duchovskis (2004), there are two periods in flowering induction and evocation. The first period of flowering induction is photoinduction (5 leaves in rosette for carrot). Metabolites of photomorphogenetic system are transported to apical meristems, where they can de-block the genes of inflorescence axis formation. From this moment evocation period first starts which ends with the formation of the inflorescence axis. The second period of flowering induction of wintering plants is thermo induction (vernalization; 9 leaves in rosette for carrot); its metabolites determine the formation of inflorescence axis structures (evocation period second). These processes requite in flower initiation and differentiation (Duchovskis 2004).

The aim of this study was to investigate the dynamics of phytohormones in carrot during different flowering initiation stages.

Materials and Methods

Carrot *Daucus carota* L. var. *Garduolė 2* was initially grown in vegetative tumbler 54x34x15 cm size placed in a greenhouse (16 h photoperiod and 21/16°C day/night temperature). Peat (pH ~6) was used as the substrate. Carrots with 5 and 9 leaves in rosette, respectively, were removed from the greenhouse and placed in phytotron chambers with different conditions for 120 days: EXP1 – photoperiod of 0 h and 4°C temperature, EXP2 – photoperiod of 8 h and 4°C temperature, EXP3 – photoperiod of 16 h and 4°C temperature,

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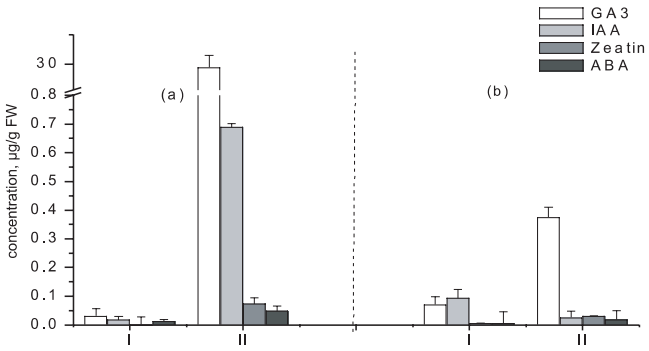

Figure 1. Changes in phytohormone concentrations in carrot rosette before (I) and after (II) flowering induction. (a) 5 leaves in rosette. (b) 9 leaves in rosette.

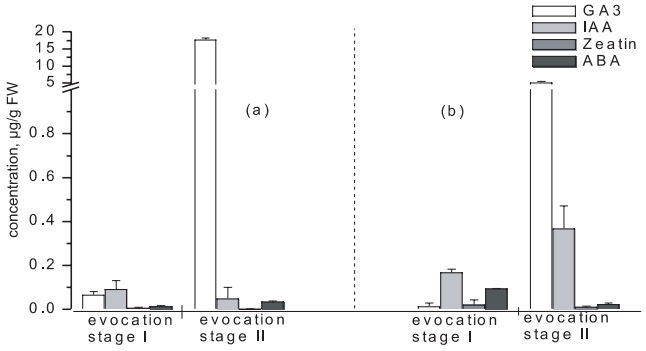

Figure 2. Changes in phytohormone concentrations in carrot during various flowering initiation stages. Photoperiod of 0 h and 4°C temperature (EXP1). (a) 9 leaves in rosette. (b) 5 leaves in rosette.

EXP4 – photoperiod of 8 h and 21/16°C temperature, EXP5 – photoperiod of 16 h and 21/16°C (day/night) temperature. After that organogenesis processes (Kuperman et al. 1982) were investigated with the photoperiod of 16 h and 21/16±2°C (day/night) temperature maintained. Plants were investigated under illumination using HPI-T lamps (Philips).

Samples for high-pressure liquid chromatography (HPLC) were prepared by grounding 1 to 2 g of fresh tissue per sample into powder under liquid nitrogen treatment. The samples were pre-purified using solid-phase extraction with NH_2-cartridge columns. The prepared samples were stored in vials at 4°C, as proposed by Wang et al. (2003).

Analysis of gibberellic acid (GA_3), indolyl-3-acetic acid (IAA), abscisic acid (ABA) and zeatin was performed using a Shimadzu HPLC model 10A chromatographer equipped with DAD detector (SPD-M 10A VP), column oven (CTO-10AS VP), degasser (DGU-14A), and two pumps (LC-10AT VP) enabling use of concentration gradient of the mobile phase. Separation and detection were performed on an Inertsil ODS-2 column (150 x 4.6 mm^2). Mobile phase gradients of 40% methanol containing 1% acetic acid for GA_3, 45% methanol in 1% acetic acid for IAA, 50% methanol in 1% acetic acid for zeatin, and 55% methanol in 1% acetic acid for ABA were used. The wavelengths of 254 nm, 280 nm, 270 nm, and 254 nm were set in the DAD detector for GA_3, IAA, zeatin, and ABA, respectively. The total run time for the separations at a flow rate of 1 mL/min was approximately 10 min.

The following chemicals were used: isopropanol (POCH, Poland), imidazole, GA_3, IAA, ABA and zeatin (Sigma-Aldrich, Germany), NH_2 -columns (Supelco, USA), methanol and hexane (LaChema, Czech Republic), acetic acid (BOH, England).

Results

The content of GA_3 and IAA dramatically increased after flowering induction, especially for carrots with 5 leaves in rosette (Fig. 1).

Under treatment with photoperiod of 0 h and 4°C temperature, the development of carrots stopped after evocation stage II. The concentration of GA_3 increased after evocation stage I in both cases, but the higher concentration was in the apex zone in carrots with 5 leaves in rosette (Fig. 2). As the concentration of GA_3 was low, the small increase of ABA was observed in evocation stage I (Fig. 2b). The content of IAA was higher in carrot with 9 than with 5 leaves in rosette.

Elements of inflorescence axis were formed (organogenesis stage IV) and further flowering initiation stages were reached faster by carrots with 9 leaves in rosette. However, the development of carrots with 9 leaves in rosette in different treatments was not identical. The growth of generative organs was the best in treatments EXP2 and EXP3, but the development of carrots with 9 leaves in rosette was faster than with 5 leaves in rosette (Table 1). The general tendency of GA_3 biosynthesis is observed in all treatments: low GA_3 levels were in evocation stage I, and the GA_3 content decreased during flower initiation (except of EXP2, carrots with 9 leaves in rosette). During flowering initiation stages the concentration of GA_3 was higher under treatment with low positive temperatures (EXP2 and EXP3) than under treatment with high temperatures (EXP4 andEXP5). Extremely high IAA concentrations were detected under treatment with high temperatures during evocation stage II in carrot with 5 leaves in rosette both in a short day (SD) or long day (LD) photoperiod (EXP4 and EXP5). The increase of IAA amount during evocation stage II and decrease during flower initiation was observed in all treatments in carrots with 5 leaves in rosette. However, the increase of IAA biosynthesis during all flowering initiation stages was observed for carrots with 9 leaves in rosette, except under treatment with low positive temperatures and LD photoperiod (EXP3). The highest ABA content was under treatment with a short day photoperiod and low positive temperatures (EXP2) in carrots with 9 leaves in rosette during evocation stage I and dramatically decreased in evocation stage II (Fig. 3).

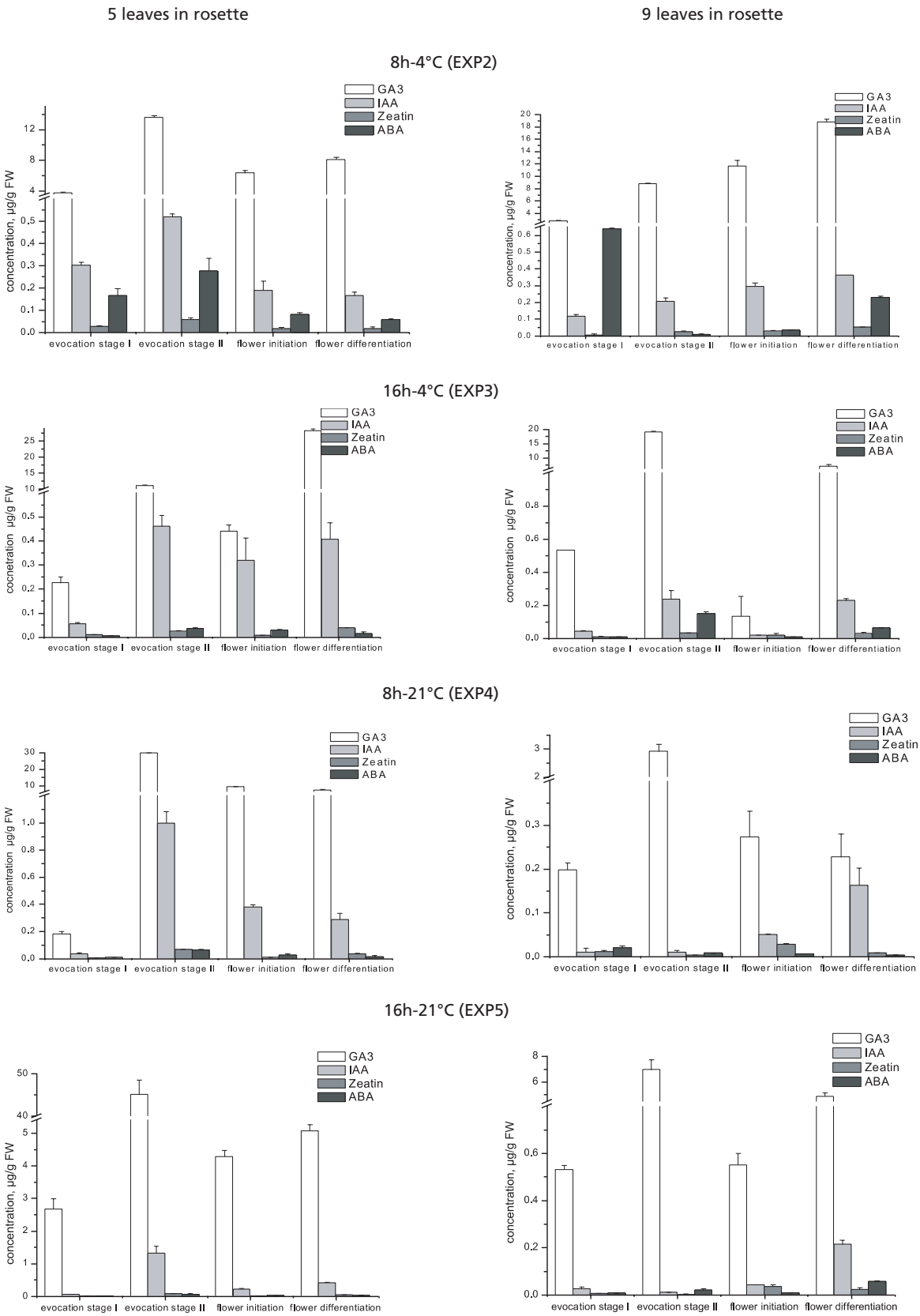

Figure 3. Changes in phytohormone concentrations in carrot under treatments with different photo and thermo periods.

Table 1. The intensity level of carrot development processes during different flowering initiation stages.

Treatment	Flowering initiation stage: Evocation stage I (organogenesis stage III) I	Evocation stage I (organogenesis stage III) II	Evocation stage II (organogenesis stage IV) I	Evocation stage II (organogenesis stage IV) II	Flower initiation (organogenesis stage V^a) I	Flower initiation (organogenesis stage V^a) II	Flower differentiation (organogenesis stage V^b, V^c) I	Flower differentiation (organogenesis stage V^b, V^c) II
EXP1	+	+	+	+	-	-	-	-
EXP2	+ + + +	+ + + + +	+ + + +	+++++	+ + + +	+++++	+ + + +	+++++
EXP3	+ + + +	+ + + + +	+ + + +	+++++	+ + + +	+++++	+ + + +	+++++
EXP4	+ +	+ + +	+ +	+ + +	+ +	+ + +	+ +	+ + +
EXP5	+ +	+ + +	+ +	+ + +	+ +	+ + +	+ +	+ + +

I - 5 leaves in rosette; II - 9 leaves in rosette.
"+/-" - development intensity level in carrot.

Discussion

As it is known, different development levels are needed for photo and thermo induction in carrots (Duchovskis et al. 2003; Duchovskis 2004). Photo- and thermo induction mechanisms are not interrelated. For the first stage of flowering induction (photo induction) 5 leaves in rosette are needed. From this moment starts the first evocation stage which ends with the formation of the inflorescence axis. After that the second evocation stage (thermo induction) starts. During these processes the formation of inflorescence axis elements is complete. At least 9 assimilating leaves are needed for the reaction to thermo induction (Duchovskis et al. 2003). The development of the investigated plants was unequal. Environmental cues are perceived by different organs in the plant and promote endogenous stimuli that signal the apical meristem (Lejeune et al. 1991). Biennial plants cannot be vernalized as imbibed seeds or young seedlings but rather must reach a critical age or developmental stage before vernalization can occur (Lang 1986). The shoot apex must be exposed to cold for vernalization to occur. This is consistent with vernalization causing the apical meristem to acquire competence to flower. In many species the plant hormone gibberellin plays a role in the regulation of flowering (Michaels and Amasino 2000). Could an increase in endogenous GA amount or an alteration of GA metabolism be a mechanism for the promotion of flowering without inductive photoperiods or cold treatment? Our results showed that an increase of GA_3 content in evocation stage II and decrease in flower initiation stage determines faster stem elongation and bud formation. Carrots under treatment with low temperatures showed the more rapid development. There is an assumption that changes in GA_3 quantity could be related to cold treatment, while changes in photoperiod did not give such results. It is known that both antagonistic as well as stimulatory steps are involved during hormonal action and the balance of these determines the final effect of even a single hormone (Johri and Mitra 2001). In several responses, such as flowering initiation, GA and ABA act antagonistically. Under treatment with SD photoperiod and low temperatures in evocation stage I the high ABA and low GA_3 concentrations were detected. After that decrease of ABA and increase of GA_3 level in evocation stage II can be related with fast flowering induction processes. Auxins are typically associated with cell elongation, while auxin and cytokinin act synergistically to regulate the process of cell division (Johri and Mitra 2001; Merkys et al. 2003). According to Duchovskis (2003, 2004), evocation period first ends with the formation of the inflorescence axis. Metabolites of the second period of flowering induction determine the formation of inflorescence axis structures (evocation period second). The common tendency of IAA biosynthesis was observed in all treatments for carrots with 5 leaves in rosette. The highest IAA concentration in evocation stage II may induce the formation of inflorescence axis structures. It seems that temperature influence has higher effect on IAA biosynthesis than differences in photoperiod.

Conclusions

1. The best rate of development was during exposure to florally inductive effect with low temperature either a short day or long day photoperiod in carrots with 9 leaves in rosette, when plants has a competency to accept influence of thermo induction.
2. Temperature influence has higher effect on phytohormones biosynthesis than differences in photoperiod.
3. It seems presumptively that the ratio of investigated phytohormones has substantial influence on flowering initiation processes in carrots:
- an increase of GA_3 content in evocation stage II and decrease in flower initiation stage determines faster stem elongation and bud formation;
- a decrease of ABA and increase of GA_3 level in evocation stage II can be related with fast flowering induction processes;
- the highest IAA concentration in evocation stage II may induce the formation of inflorescence axis structures.

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