Flowering initiation in plants of different *Apiaceae* species

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Abstract

In this paper the action of flowering initiation is described as a multicomponent and multistep mechanism. This, without other endogenous and exogenous factors, depends on solid action of photosynthesis, its primary metabolites and phytohormones. Our hypothesis is based on multifunctional flowering initiation model of biennial plants. The aim of this paper was to investigate the processes of juvenile period and flowering initiation; to evaluate the role of photosynthetic system; to describe the balance of phytohormones and sugars during morphogenesis in edible carrot (*Daucus sativus* (Hoffm.) Röhl.) and common caraway (*Carum carvi* L.). It was determined that the end of juvenile period in both carrot and caraway ontogenesis is related to formation of 9 assimilated leaves and with full competence to accept the stimulus of photo and thermo induction. Cold requirement for flower initiation in caraway is facultative therefore vernalization is not the main factor which strictly controls floral development. Active action of photosynthetic system and release of assimilates during evocation processes before flower initiation highlights the role of assimilates (especially of sucrose) in flowering initiation processes of *Apiaceae* plants. Synergistic interaction of ABA/GA₃ and zeatin/IAA and antagonistic interaction of ABA/IAA determined the formation of flowering stem and flowering axis structures; however the development intensity depended on thermo and photoperiodic conditions.

Key words: carbohydrates, chlorophylls, evocation, phytohormones, *Apiaceae*.

Introduction

The *Apiaceae* are mostly temperate herbs almost always with umbellate inflorescences comprising about 300 genera and 3,000 species /Pimenov, Leonov, 1993/. *Daucus carota* subsp. *sativus* (Hoffm.) Arcang., the common cultivated carrot, is by far its most economically important member /Downie et al., 2000/. Other familiar vegetables, flavourings or garnishes include angelica, caraway, celery, dill, parsley etc.

Factors, which may induce flowering in biennial plants, are the complex ones. Differences in the vegetation length and induction requirement have their genetical and physiological background /Németh, 1998/. The genetical code regulates the physiological processes, through which the flower development takes place. According to
Kuckuck et al. (1988), a flexible adaptation potential to different ecological circumstances, first of all temperature and photoperiod is assured by cross pollination.

In a number of species, cold effect is the major factor stimulating flower initiation. Its value and length are of basic importance; however, they are satisfactorily cleared up only for a few species /Németh, 1998/. The required vernalization length is in connection with the development and size of the plants, more developed ones demanding a shorter period. Also other factors are involved, in several cases the interaction of temperature and photoperiods are proved /Ramin, Atherton, 1994/. For the majority of the most important vegetables of the Apiaceae family temperatures between +5–10°C proved to be the most effective, however both lower (+5°C) and higher (+15°C) temperatures might have an inductive effect /Rünger, 1977/. Optimal length of the inductive period however, seems to be different not only for each species but also differs among varieties. An effective vernalization period among varieties of Daucus carota were shown by Dias-Tagliacozzo and Valio (1994), where cultivar ‘Nantes’ required at least 3 months of inductive temperatures, for ‘Brasilia’ 5 days proved to be sufficient. According to practical observations of Toxopeus and Bouwmeester (1993) biennial caraway varieties need about 8 weeks’ vernalization period at low temperatures below +10°C. It seems that vernalization circumstances are characteristic not only for species but also for interspecific taxa.

Besides temperature, illumination length may also play a basic role in flower initiation /Rünger, 1977/. Those inductive factors stay in tight correlation with each other /Booij, Meurs, 1994/. Illumination may act on flowering through its length during the day (the ratio of light to dark hours is important), its length during the plant life and sometimes its intensity /Németh, 1998/. In the case of caraway, scientific data on photoperiodic reaction are very few. According to Putievsky (1983), caraway does not need any special photoperiod for flowering.

The aim of this paper was to investigate the processes of juvenile period and flowering initiation; to evaluate the role of photosynthetic system; to describe the balance of phytohormones and sugars during morphogenesis in edible carrot and common caraway.

**Materials and methods**

The researches were carried out during 2004–2007 in a phytotron complex of LIH according to vegetative assay methodology /źubricki, 1974/. Edible carrot (Daucus sativus (Hoffm.) Röhl.) var. ‘Garduolės’ and common caraway (Carum carvi L.) var. ‘Gintaras’ were initially grown in a pot, 54 x 34 x 15 cm in size, placed in a greenhouse until particular developmental level needed for special experiment (16-hour photoperiod and +21/16°C day/night temperature). Peat (pH ≈ 6) was used as a substrate. Plants were fertilized with 0.2% ammonium nitrate solution once a week.

Carrots with 5 and 9 leaves in a rosette and caraway with 7 and 9 leaves in a rosette were kept in the phytotron chambers with different photo and thermo periods for 120 days: 0 h – +4°C, 8 h – +4°C, 16 h – +4°C, 8 h – +21/17°C, 16 h – +21/17°C. Then evocation, flower initiation and differentiation processes were investigated under illumination with the photoperiod of 16-hour and +21/16 ± 2°C day/night temperatures. The following parameters were determined: organogenesis stage /Куперман и др., 1982/;
flowering initiation stage /Duchovskis, 2000/ which was estimated by the intensity level of new structures formation; the photosynthetic pigment contents (spectrophotometrically) /Гавриленко, Жыгалова, 2003/; phytohormones and sugars by HPLC method. Limits of detection for phytohormones: gibberellic acid (GA_3) 0.87 µg ml^{-1}, indol-3-acetic acid (IAA) 0.03 µg ml^{-1}, zeatin 0.49 µg ml^{-1} and abscisic acid (ABA) 0.18 µg ml^{-1} /Wang et al., 2003/. Limits of detection for carbohydrates: fructose 0.01 µg ml^{-1}, glucose 0.12 µg ml^{-1}, sucrose and maltose 0.02 µg ml^{-1} /Urbonavičiūtė et al., 2006/. The standard deviation was calculated using MS Excel software (version 7.0).

**Results and discussion**

In this paper the action of flowering initiation is described as a multicomponent and multistep mechanism. Our hypothesis of flowering initiation is based on multifunctional flowering initiation model of biennial plants. Despite other endogenous and egzogenous factors it depends on solid action of photosynthesis, its primary metabolites and phytohormones. The biology of carrot and caraway is different due to their morphology and metabolism therefore it is interesting to compare flowering initiation pathways in these plants. Plant flowering initiation processes are related to the duration of juvenile period. During this period plants are insensitive to any inductive factor and are unable to form reproductive organs. Confirming the conception of two stages flowering induction and evocation of wintering plants, we assume that the mechanisms of photo and thermo induction in edible carrot are independent and autonomous. That is why the minimal developmental level to accept these flowering inductive factors differs /Dias-Tagliacozzo, Valio, 1994; Duchovskis et al., 2003/. According to our previous data, the inflorescence axis was formed only when plants had 5–6 leaves in a rosette. The formation of inflorescence axis means that photo induction ended and after that the processes of first evocation stage began /Duchovskis, 2000/. The photo induction conditioned the formation of inflorescence axis elements (IV organogenesis stage) when carrots had 8–9 leaves in rosette /Duchovskis et al., 2003/. Therefore, modulating the flowering initiation processes, carrots were placed into inductive regime with 5 and 9 leaves in a rosette, respectively. The best developmental rate was observed in carrots with 9 leaves in a rosette. However, unlike high temperature, low positive temperature caused faster developmental rate independently from duration of photoperiod. Under dark conditions carrots and caraway did not develop and rooted away (Table). As for caraway, it seems that juvenile period is longer than in carrots. According to Rünger (1977), both lower (+5°C) and higher (+15°C) temperatures might have an inductive effect, which agrees with our results. Neither different duration of photoperiod nor vernalization or high (+21/17°C) temperature caused the formation of generative organs in caraway with 7 leaves in a rosette; they remained in a vegetative stage.
**Table.** The intensity level of different flowering initiation stages in edible carrot and common caraway

**Lentelė.** Valgomasios morkos ir paprastojo kmyno skirtingų žydėjimo iniciacijos tarpsnių intensyvumo lygis

<table>
<thead>
<tr>
<th>Treatment Poveikis</th>
<th>Flowering initiation stages / Žydėjimo iniciacijos tarpsniai</th>
<th>Flower initiation and differentiation / Žiedų iniciacija ir diferenciacija</th>
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<tbody>
<tr>
<td></td>
<td>I and II evocation stages (III and IV organogenesis stages)</td>
<td>(V&lt;sup&gt;a&lt;/sup&gt; and V&lt;sup&gt;b&lt;/sup&gt; organogenesis stages)</td>
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<td>I ir II evokacijos tarpsniai (III ir IV organogenezės tarpsniai)</td>
<td>Žiedų iniciacija ir diferenciacija (V&lt;sup&gt;a&lt;/sup&gt; ir V&lt;sup&gt;b&lt;/sup&gt; organogenezės tarpsniai)</td>
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<tr>
<td>Edible carrot / Valgomoji morka</td>
<td>5 leaves / 5 lapai</td>
<td>9 leaves / 9 lapai</td>
</tr>
<tr>
<td>0 h +4°C</td>
<td>1</td>
<td>1</td>
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<tr>
<td>8 h +4°C</td>
<td>4</td>
<td>5</td>
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<td>16 h +4°C</td>
<td>4</td>
<td>5</td>
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<tr>
<td>8 h +21/17°C</td>
<td>2</td>
<td>3</td>
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<td>16 h +21/17°C</td>
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<tr>
<th>Common caraway / Paprastasis kmynas</th>
<th>7 leaves / 7 lapai</th>
<th>9 leaves / 9 lapai</th>
<th>7 leaves / 7 lapai</th>
<th>9 leaves / 9 lapai</th>
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<tr>
<td>0 h +4°C</td>
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<td>8 h +4°C</td>
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<td>16 h +4°C</td>
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<tr>
<td>16 h +21/17°C</td>
<td>–</td>
<td>3</td>
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Note. 1 point – the lowest development rate, 5 points – the most intensive.

Pastaba. 1 balas – lėčiausias vystymosi lygis, 5 balai – intensyviausias.

Vernalization and long day (unlike short day) influenced the most intensive formation of generative organs in caraway with 9 leaves in a rosette. Meanwhile under high (+21/17 °C) temperature the duration of photoperiod did not cause any restriction of caraway flowering rate (Table). Németh (1998) noticed that optimal induction regime for caraway might lie between +5°C and +8°C, which is effective when lasting more than two weeks. Both a shorter period as well as high temperatures result in partial flowering. In the case of caraway, scientific data are very few. Putievsky (1983) examined the effect of day length and temperatures on the flowering of three Apiacea species: caraway, dill and coriander. The tree spices exhibited different reactions to the treatments. Caraway developed flowers under all experimental circumstances (+18/12°C or +24/12°C day and night temperatures, with 10 or 16 h photoperiods). Pursuant to other authors a longer vegetative growth at lower (+4°C) temperature and short day (8 h) occurred, whereas earlier flowering was preceded by long day (16 h) and low temperature. The duration of photoperiod did not affect flowering rate under treatment with high temperature (Table). It might mean, either that caraway does not need any short day induction for flower initiation at all, or any photoperiodic response is effective only with interaction of low temperatures /Németh, 1998/. The question is: might it
happen, that biennial varieties do not require a definite cold period at all, winter only stops their development for a while?

The participation of photosynthetic system and their metabolites in flower initiation processes is still unclear. How do phytohormones, carbohydrates and other materials distribute and interact during photo and thermo induction and during other flowering initiation processes?

In photoperiodically sensitive plants, the flowering signals are translocated from the leaves to the apex. The meristem supply by sugars during floral induction can be obtained as result of photosynthesis or it can come from starch metabolism /Eshghi, Tafazoli, 2006/. According to our data, it was determined that in both carrot (independently from development level) and in caraway, under treatment with low (+4°C) temperature the increase in the amount of photosynthetic pigments was insignificant, though under treatment with high (+21/17°C) temperature the decrease of the photosynthetic pigments concentration was observed (Fig. 1 A, B and C). Also in both carrot and caraway, during I and II evocation stages the changes in amounts of photosynthetic pigments were unremarkable (Fig. 1 A, B and C). The highest concentrations of chlorophyll \(a\), \(b\) and carotenoids were accumulated in carrots with 9 leaves in rosette (Fig. 1 B) and in caraway (Fig. 1 C) under treatment with low temperature after evocation, during flower initiation stage. Exactly in these treatments (8 h – +4°C, 16 h – +4°C) the development rate in carrots and in caraway (16 h – +4°C) was the best (Table). The lowest concentrations of photosynthetic pigments in caraway leaves were determined during flower differentiation stage in all treatments (Fig. 1 C).

Analysing the changes in chlorophyll \(a\) to \(b\) ratio in carrots it was noticed that the most stable ratio (3.54–3.00) was in carrots with 9 leaves in a rosette during all flowering initiation stages (Fig. 1 E). The highest chlorophyll \(a\) to \(b\) ratio (4.00–3.49) was in carrots with 5 leaves in a rosette in all treatments which slightly decreased after I evocation stage (Fig. 1 D). Analysing data in caraway it was noticed that low temperature and short day affected the largest variation in chlorophyll \(a\) to \(b\) ratio. Under these conditions the ratio decreased till 2.1 during flowering initiation stage (Fig. 1 F). The most stable (3.1) chlorophyll \(a\) to \(b\) ratio was determined during evocation and flower initiation under treatment with high (+21/17°C) temperature and short day (8 h). The highest ratio of these pigments was observed during flower differentiation in all treatments (Fig. 1 F).

The sugar response pathway is very complicated and interacts with other plant metabolic pathways. Presumably active synthesis of photosynthetic pigments determines good accumulation of metabolites (sugars), which induce further developmental processes and compose the mechanism that regulates plant development and flowering /Borisjuk et al., 2003; Gibson, 2005/. Bouwmeester and Kuijpers (1993) in experiments with annual and biennial caraway in field and in greenhouse conditions showed that fruit formation, number and growth is more dependent on carbohydrate assimilates rather than on genetic composition. According to our data, high glucose concentrations were determined in apical meristems of carrots with 5 leaves in a rosette during evocation, which decreased during flower initiation and differentiation (Fig. 2 A). This means that glucose can act as morphogenetic factor. A lot of scientists investigated the sucrose distribution in apex and in other plant tissues /Chailackhyan, 1936; Bodson, Outlaw, 1985;
Edible carrot / Valgomoji morka
5 leaves / 5 lapai

- Evocation stage I
- Evocation stage II
- Flower initiation
- Flower differentiation

Common caraway / Paprastasis kmynas
9 leaves / 9 lapai
Figure 1. The amount (A, B, C) and ratio (D, E, F) of photosynthetic pigments in leaves of edible carrot and common caraway during different periods of flowering initiation

Edible carrot / Valgomoji morka
5 leaves / 5 lapai

Common caraway / Paprastasis kmynas
9 leaves / 9 lapai

*Figure 1.* Fotosintežės pigmentų kiekis (A, B, C) bei santykis (D, E, F) valgomojos morkos ir paprastojo kmyno laipuose skirtingais žydem jimo iniciacijos tarpsniais.
Figure 2. The amount (A, B, C) of carbohydrates in apical meristems of edible carrot and common caraway during different periods of flowering initiation.

2 paveikslas. Sacharidų kiekis (A, B, C) valgomoji morkos ir paprastojo kmyno apikalinėse meristemose skirtingais žydėjimo iniciacijos tarpsniais.
**Edible carrot / Valgomoji morka**

5 leaves / 5 lapai

**Common caraway / Paprastasis kmynas**

9 leaves / 9 lapai

**Figure 3.** The ratio (A, B, C) of phytohormones in apical meristems of edible carrot and common caraway during different periods of flowering initiation

3 paveikslas. Fitohormonų santykis (A, B, C) valgomosios morkos ir paprastojo kmyno apikalinėse meristemose skirtingais žydėjimo iniciacijos tarpsniais
It is presumed that the supply of sucrose to apical meristemic tissues is important for flower induction. Still it may not be the specific flowering induction stimulus and independent from the response to the photoperiod duration. In agreement with other authors /Borisjuk et al., 2002/, the highest sucrose concentrations were determined in cells which can actively divide (Fig. 2 A). Carrots with 9 leaves in a rosette can accept the stimulus of photo induction. Unlike caraway (Fig. 2 C), during the first and second evocation stages and during flower initiation, high temperature disturbs sugar metabolism in carrot apical meristems (Fig. 2 B). Such sugar metabolism and transport to apical meristems can determine the differences in plant development processes (Table). Also it may depend on the special plant requirement to photo and thermo induction for the acception of flowering stimulus.

It is known that many plant developmental, physiological and metabolic processes are regulated by soluble sugars such as glucose and sucrose and by other signalling molecules, such as phytohormones /Gibson, 2004/. The response pathway between sugar and phytohormone interaction in some cases might ‘interact’ or exhibit ‘crosstalk’ in the regulation of many processes. One mechanism by which sugars affect phytohormone response is by altering the levels, localization and/or transport of different phytohormones. In many works it is stated that hexoses affect phytohormone metabolism and/or transport /Radley, 1969; Toyofuku et al., 2000; Cheng et al., 2002; Gibson, 2004/. It is known that abscisic acid plays an important role during mitotic activity. Contrary to antagonistic action of ABA and glucose during early development, ABA and sucrose during further development stages stimulates the accumulation of assimilates /Finkelstein, Gibson, 2002/. According to our results such tendencies of ABA and mentioned sugars were determined under all flowering induction conditions especially for carrots (Fig. 2 and 3). It might be that an interaction between sugars and phytohormones pathways depends not only on developmental level of the plant but also on ability to accept the flowering stimulus. Analysing the ratio of phytohormones in apex of both carrot and caraway synergistic or very week antagonistic action of abscisic and gibberellic acids was determined in all treatments (Fig. 3 A, B and C). Under treatment with short day (8 h) and low temperature (+4°C) stronger antagonistic (0.54–0.35) action of abscisic and indolil-3-acetic acids was observed in carrot with 5 leaves in a rosette. Notwithstanding stronger synergistic (about 0.1) action of zeatin and indolil-3-acetic acid asserted under these conditions (Fig. 3 A). Thus the ratio of auxin and cytokinin determines the differentiation of cells /Frank et al., 2000/. Such sucrose (Fig. 2) and gibberellic acid (Fig. 3) concentration increase during II evocation stage before flower initiation, shows general interaction of metabolic processes which are involved in flower formation. In apical meristems of carrot with 9 leaves in a rosette, the stronger antagonistic action of ABA/IAA and weaker synergistic action of zeatin/IAA was observed in II evocation stage, contrary to flower initiation stage (Fig. 3 B). The same tendency in variation of ABA/IAA and zeatin/IAA ratio during II evocation stage and flower differentiation in common caraway was observed (Fig. 3 C). Therefore such action induces the elongation of flowering stem and mitotic activity of apical meristems. Besides, determined antagonistic action of ABA/IAA suppresses an apical dominance, thus the formation of lateral structures occurs.
Conclusions

1. The end of juvenile period in both carrot and caraway ontogenesis is related to the formation of 9 assimilated leaves and to full capacity to accept the stimulus of photo and thermo induction.

2. Cold requirement for flower initiation in caraway is facultative, therefore vernalization is not the main factor which strictly controls floral development.

3. Active action of photosynthetic system and release of assimilates during evocation processes before flower initiation highlight the role of assimilates (especially of sucrose) in flowering initiation processes of Apiaceae plants.

4. Synergistic interaction of ABA/GA3 and zeatin/IAA and antagonistic interaction of ABA/IAA determined the formation of flowering stem and flowering axis structures, however the development intensity depended on thermal and photoperiodic conditions.

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Skirtingų Apiaceae rūšių augalų žydėjimo iniciacija

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Santrauka

Straipsnyje žydėjimo iniciacija apibūdinama kaip daugiakomponentis ir daugiapakopis mechanizmas, be daugelio kitų išorinių ir vidinių veiknių, priklausantis nuo glaudžios fotosintezės, pirminių metabolitų bei fitohormonų sąveikos. Hipotezė grindžiama daugiafunkciu dvimečių augalų žydėjimo iniciacijos modeliu. Darbo tikslas – ištirti juvenalinio periodo procesus ir žydėjimo iniciaciją, įvertinti fotosintetinės sistemos veiklą, išanalizuoti fitohormonų ir cukrų balansą valgomasios morkos (Daucus sativus (Hoffm.) Röhl.) ir paprastojo kmyno (Carum carvi L.) morfogenezės metu. Nustatyta, kad esant ir morkų, ir kmynų ontogenezei juvenalinio periodo pabaiga susijusi su 9 asimiliuojančių lapų suformavimu. Tuo metu šie augalai yra visiškai pasiruošę priimti foto- ir termoindukcijos stimulus. Kmynų žiedų iniciacijos šalčio poreikis yra fakultatyvus, todėl vernalizacija nėra pagrindinis žiedų vystymosi kontroliuojantis veiksnys. Aktyvi fotosintetinės sistemos veikla ir asimiliatajų atjungimas evokacijos proceso metu prieš žiedų iniciaciją pabrėžia asimiliatų (ypač sacharozės) įtaką Apiaceae šeimos augalų žydėjimo iniciacijos procesams. Žiedyniebieė ir žiedyno ašies struktūrų formavimą lėmė sinergetinė ABA bei GA₃ ir zeatino bei IAA sąveika, o vystymosi intensyvumas priklausė nuo termo- ir fotoperiodo sąlygų.

Reikšminiai žodžiai: angliavandeniai, chlorofilai, evokacija, fitohormonai, Apiaceae.