

## Flowering initiation in carrot and caraway

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The aim of this paper was to analyze the influence of external factors such as short and long day, vernalization and temperature on the generative development rate in biannual plants and on sucrose and gibberelic acid metabolism in apical meristems during flowering initiation. The researches were carried out in 2004–2007 in phytotron complex of the Lithuanian Institute of Horticulture according to vegetative assay methodology under controlled conditions. Edible carrot (*Daucus sativus* (Hoffm.) Röhl.) var. ‘Garduolės’ and common caraway (*Carum carvi* L.) var. ‘Gintaras’ with 9 leaves in rosette were kept in a 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. Different developmental rates and ways in two disputed *Apiaceae* species were observed in subject to environmental factors. Thus the peculiar sucrose supply in shoot apex and differences in GA<sub>3</sub> concentration during evocation under particular environmental conditions influenced the formation rate of inflorescence stem in carrot and caraway. We deduced that vernalization makes stronger positive effect on carrot flowering initiation, whereas high temperature blocks the formation of generative organs. The flowering initiation in carrots is more dependent on temperature than on photoperiod regimes during different ontogenesis stages. Long day and vernalization determines almost full flowering, high temperatures independently from photoperiod results in partial flowering and short day and vernalization is the limiting factor of caraway flowering.

**Key words:** caraway, carrot, gibberellic acid, photoperiod, sucrose, temperature, vernalization.

**Introduction.** The *Apiaceae* are mostly temperate herbs almost always with umbellate inflorescences comprising about 300 genera and 3,000 species (Pimenov and 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, flavorings or garnishes include angelica, caraway, celery, dill, parsley and etc.

The timing of the transition from vegetative growth to flowering is of paramount importance in agriculture, horticulture, and plant breeding because flowering is the first step of sexual reproduction (Bernier et al., 1993). Factors, which may induce flowering in biennial plants, are complex ones. Differences in the vegetation length and induction requirement have their genetical and physiological background (Németh, 1998). In

several 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 and Atherton, 1994). Beside 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 and Meurs, 1994). Illumination may act on flowering through its length during the day (the ratio of light and dark is important), its length during the plant life and sometimes its intensity (Németh, 1998). The different flowering-promoting factors are perceived by different parts of the plant. Temperature is perceived by all plant parts, vernalization mainly by the shoot apex, and photoperiod – by matured leaves. Therefore, this implies that these parts interact and that the fate of the apical meristem – remaining vegetative or becoming reproductive – is controlled by an array of long-distance signals from the entire plant (Bernier et al., 1993). The transport of sucrose from leaves to apical meristem (Bernier et al., 1993) and increase in gibberellins concentration is observed during flowering induction (Blázquez et al., 1998). Multiple lines of evidence indicate that many of plant developmental and physiological processes are regulated in response to other signaling molecules, such as sucrose or gibberellins (Gibson, 2004). Therefore, the theory was raised that flowering initiation acts as multicomponent and multistep mechanism and without other endogenous and exogenous factors, depends on solid action of phytohormones and sugars (Bernier, 1988).

The aim of this paper was to analyze the influence of external factors such as short and long day, vernalization and temperature on the generative development rate in biannual plants and on sucrose and gibberellic acid metabolism in apical meristems during flowering initiation.

**Object, methods and conditions.** The researches were carried out during 2004–2007 in phytotron complex of the Lithuanian Institute of Horticulture according to vegetative assay methodology (Zubricki, 1974). Edible carrot (*Daucus sativus* (Hoffm.) Röhl.) var. ‘Garduolės’ and common caraway (*Carum carvi* L.) var. ‘Gintaras’ were initially grown in vegetative tumbler, 54 × 34 × 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) was reached. Peat (pH ≈ 6) was used as a substrate.

Carrots and caraway with 9 leaves in rosette were kept in a 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. After the exposure, evocation, flower initiation and differentiation processes were investigated under illumination with the photoperiod of 16-hour and 21/16 ± 2 °C day/night temperatures. Determined parameters: organogenesis stage (Куперман, 1982); flowering initiation stage (Duchovskis, 2000). Analyses of gibberellic acid (GA<sub>3</sub>) were performed using a Shimadzu HPLC model 10A chromatographer equipped with DAD detector (SPD-M 10A VP), the detection wavelength – 254 nm. Separations were performed on an Inertsil ODS-2 column (150 × 4.6 mm<sup>2</sup>). Mobile phase: of 45 % methanol

containing 1 % acetic acid. Analyses of sucrose were performed on the same Shimadzu HPLC model 10A equipped with refractive index detector (RID 10A). Separations were performed on an Adsorbosil NH<sub>2</sub>-column (150 mm × 4.6 mm). Mobile phase: 75 % acetonitrile. Limits of detection: for GA<sub>3</sub> 0.87 µg ml<sup>-1</sup>, for sucrose 0.05 µg ml<sup>-1</sup>. Statistical analysis was performed using Excel (version 7.0). The data presented in figures are given as the mean ± standard error.

**Results.** As it is shown in Table, the best developmental rate was observed in carrots. In opposite to high temperature, low positive temperature caused faster development rate independently from duration of photoperiod. Under dark conditions carrots and caraway didn't develop and rooted away. Vernalization and long day (in opposite to short day) influenced the most intensive formation of generative organs in caraway. Meanwhile under high (21/17 °C) temperature the duration of photoperiod didn't cause any restriction of caraway flowering rate (table).

**Table.** The intensity level of different flowering initiation stages in common caraway and edible carrot

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

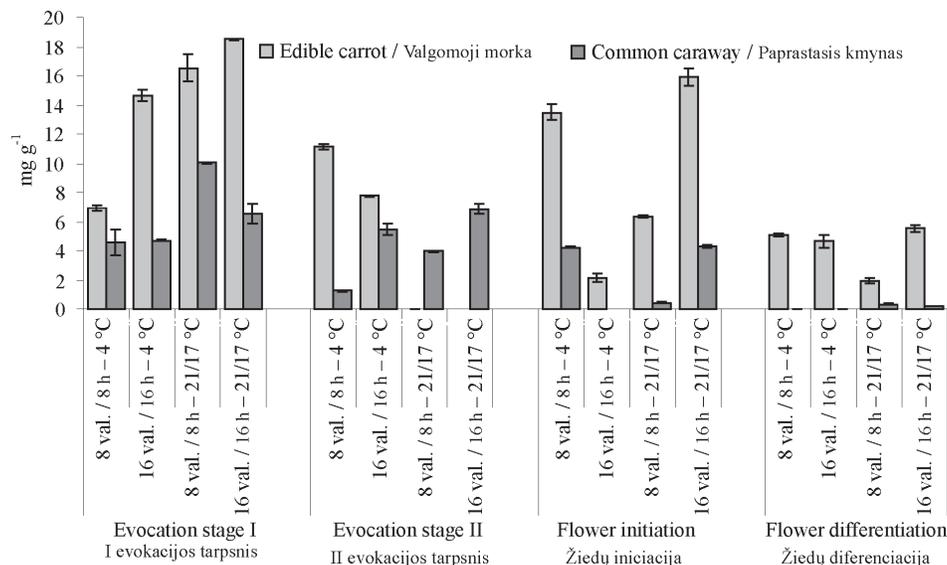
Treatment Veiksnių deriniai	Flowering initiation stages Žydėjimo iniciacijos tarpsniai							
	I <sup>st</sup> evocation stage (III <sup>rd</sup> organogenesis stage) I evokacijos tarpsnis (III organogenezės etapas)		II <sup>nd</sup> evocation stage (IV <sup>th</sup> organogenesis stage) II evokacijos tarpsnis (IV organogenezės etapas)		flower initiation (V <sup>a</sup> organogenesis stage) žiedų iniciacija (V <sup>a</sup> organogenezės etapas)		flower differentiation (V <sup>b</sup> organogenesis stage) žiedų diferenciacija (V <sup>b</sup> , V <sup>c</sup> organogenezės etapas)	
	edible carrot valgomoji morka	common caraway paprastasis kmynas	edible carrot valgomoji morka	common caraway paprastasis kmynas	edible carrot valgomoji morka	common caraway paprastasis kmynas	edible carrot valgomoji morka	common caraway paprastasis kmynas
0 h – 4 °C	1	-	1	-	-	-	-	-
8 h – 4 °C	5	2	5	2	5	2	5	2
8 h – 4 °C	5	4	5	4	5	4	5	4
8 h – 21/17 °C	4	3	4	3	4	3	4	3
16 h – 21/17 °C	4	3	4	3	4	3	4	3

*Note:* 1 point – the lowest development rate, 5 points – the most intensive

*Pastaba:* 1 balas – lėčiausias vystymasis, 5 balai – intensyviausias vystymasis

Under treatment with high (21/17 °C) temperature, independent from photoperiod, there was no sucrose detected after evocation in carrot apical meristems. Still the amount of sucrose increased during flower initiation especially under treatment with long day (16 h) (Fig. 1). While in caraway the concentration of sucrose was higher under long day (16 h) than under short day (8 h), treatment was independent from temperature regime during evocation stage II (Fig. 1). During flower initiation it decreased under treatment with high temperature. During flower initiation and differentiation under treatment with long day (16 h) and low (4 °C) temperature there was no sucrose detected in caraway apical meristems. Besides, no

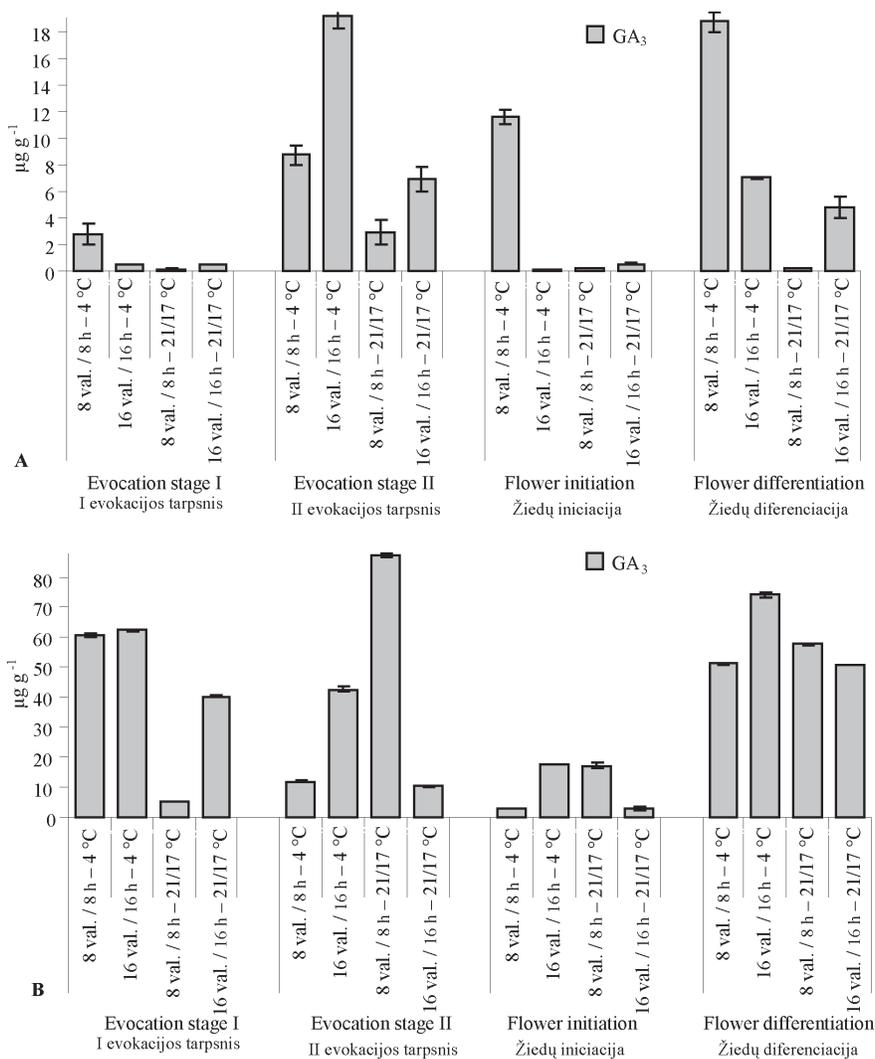
sucrose (8 h – 4 °C; 8 h – 4 °C), or very low concentrations (8 h – 21/17 °C; 16 h – 21/17 °C) were detected in caraway during flower differentiation (Fig. 1). Also the decrease in sucrose amount was observed in carrot apical meristems during this period (Fig. 1).



**Fig 1.** The amount of sucrose in apical meristems of edible carrot and common caraway during different periods of flowering initiation

**1 pav.** Sacharozės kiekis valgomosios morkos ir paprastojo kmyno apikalinėse meristemose skirtingais žydėjimo iniciacijos tarpsniais

Higher concentrations of GA<sub>3</sub> were accumulated in caraway than in carrot apical meristems. Nevertheless, analysing data in carrot apical meristems (see Fig. 2 A) it was noticed that short day (8 h) and vernalization promoted higher accumulation of gibberellic acid. The increase in gibberellic acid concentration was observed during all flowering initiation stages under these conditions (8 h – 4 °C). The highest GA<sub>3</sub> amount (19.14 µg g<sup>-1</sup>) was detected during second evocation stage under long day (16 h) and vernalization treatment. During flowering initiation the amount of GA<sub>3</sub> was lower than the limit of detection under all conditions except short day and low temperature treatment where it increased during flower differentiation (Fig. 2 A). The same downtrend in GA<sub>3</sub> concentration was observed in caraway (see Fig. 2 B) during flowering initiation; it also dramatically increased during flowering differentiation. Short day and low temperature as well as long day and high temperature (in opposite of 16 h – 4 °C and 8 h – 21/17 °C) caused low GA<sub>3</sub> accumulation in caraway apical meristems during second evocation and flower initiation periods. The increase in GA<sub>3</sub> concentrations was observed during flower differentiation under all conditions (Fig. 2 B).



**Fig. 2.** The amount of gibberellic acid (GA<sub>3</sub>) in apical meristems of edible carrot (A) and common caraway (B) during different periods of flowering initiation 2 pav. Giberelo rūgšties (GA<sub>3</sub>) kiekis valgomosios morkos (A) ir paprastojo kmyno (B) apikalinēse meristemose skirtingais žydėjimo iniciācijas tarpsniais

**Discussion.** During juvenile period plants are insensitive to any flowering inductive factor and aren't able to form reproductive organs. The minimal developmental level to accept photo and thermo inductive factors for flowering induction differs (Duchovskis et al., 2003). The formation of inflorescence axis (5 formed leaves in rosette for carrots) means that photo induction ended and after that the processes of second evocation stage began (Duchovskis, 2000). The thermo induction conditioned the formation of inflorescence axis elements (IV<sup>th</sup> 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 9 leaves in rosette when plants were able to accept both stimulus of photo and thermo induction. However, in opposite to high temperature, low positive temperature caused faster development rate independently from duration of photoperiod (Table). As for caraway, it seems that juvenile period is longer than in carrots. According to Rønger (1977), for the majority of the most important vegetables of the *Apiacea* family temperature between 5–10 °C proved to be the most effective for flowering, however both lower (5 °C) and higher (15 °C) temperatures might have an inductive effect.

E. Németh (1998) noticed that caraway optimal induction regime 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 results in partial flowering. In 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 h 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 didn't 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 that any photoperiodic response is effective only with interaction of low temperatures (Németh, 1998).

In agreement with other authors (Borisjuk et al., 2002), the highest sucrose concentrations were determined in cells which can actively divide straight before VI<sup>th</sup> organogenesis stage, when the formation of inflorescence axis elements begins (Fig. 1). Carrots with 9 leaves in rosette can accept the both stimulus of photo and thermo induction. According to our data (Fig. 1), during second evocation stage, high temperature disturbs sugar metabolism in carrot but not in caraway apical meristems. Such sugar metabolism and transport to apical meristems, influenced by photo and thermo periods, could determine the differences in plant development processes (Table). A lot of scientists investigated the sucrose distribution in apex and in other plant tissues (Chailackhyan, 1936; Bodson, 1997; Bodson, Outlaw, 1985; Lejeune et al., 1993; King, Ben-Tal, 2001). It is presumed that the supply of sucrose to apical meristemic tissues is important for flower induction. Although it is not the specific flowering induction stimulus, it is independent from the response to the photoperiod duration.

Analysing the distribution of gibberellic acid in carrot apex and the flowering effects it was noticed that short day (8 h) and vernalization conditioned constant increase in GA<sub>3</sub> amount during all flowering initiation stages (see Fig. 2 A). The same trend was observed in sucrose accumulation in carrot apical meristems (Fig. 1). Such increase in sucrose and gibberellic acid concentrations shows the common action of metabolic processes, which induce flower formation. Furthermore, the GA regulated increased sucrose transport to apex could be not short-term (King, Ben-Tal, 2001). Under these conditions (8 h – 4 °C) the fastest carrot development rate was observed (table). Regrettably, such correlation between photo and thermo

stimulus and sucrose and GA<sub>3</sub> accumulation wasn't observed in caraway apical meristems (Fig. 1; Fig. 2 B). An increase in GA<sub>3</sub> concentration under special inductive conditions was observed in both carrot (8 h – 4 °C, 16 h – 4 °C) and caraway (16 h – 4 °C, 8 h – 21/17 °C) apical meristems before flower initiation and it may be connected with developmental rate of these plants (Fig. 2, Table). Eriksson with colleagues (Eriksson et al., 2006) in experiments with *Arabidopsis* show that during growth in short days shoot apical levels of active gibberelins and sucrose increase dramatically before floral initiation occurs and that the expression patterns of the genes involved in GA metabolism suggests that this increase in GAs possibly originates from sources outside the shoot apex. Reeves and Coupland (2001) also maintained that GAs play a central role in the control of flower initiation under short days, a role that is much less important under long days, in which the flowering is delayed. As it was mentioned, different developmental rates and ways in two disputed *Apiaceae* species were observed subject to environmental factors, thus determined peculiar accumulation of sucrose and GA<sub>3</sub> in apical meristems during flowering initiation.

**Conclusions.** 1. Vernalization makes stronger positive effect on carrot flowering initiation, whereas high temperature blocks the formation of generative organs. The flowering initiation in carrots is more dependent on temperature than on photoperiod regimes during different ontogenesis stages.

2. Long day and vernalization determines almost full flowering, high temperatures independently from photoperiod results in partial flowering and short day and vernalization is the limiting factor of caraway flowering.

3. The sucrose supply in shoot apex and differences in GA<sub>3</sub> concentration during evocation under particular environmental conditions influenced the formation rate of inflorescence stem in carrot and caraway.

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Parengta spausdinti 2008 04 17

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## Žydėjimo iniciacija morkose ir kmynuose

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### *Santrauka*

Šio darbo tikslas – ištirti išorinių faktorių, kaip trumpos ir ilgos dienos, vernalizacijos ir temperatūros įtaką dvimečių augalų generatyviniame išsivystymo tempui bei sacharozės ir giberelo rūgšties metabolizmui apikalinėse meristemose žydėjimo iniciacijos metu. Tyrimai atlikti 2004–2007 metais Lietuvos sodininkystės ir daržininkystės instituto fitotroniniame komplekse pagal vegetacinių bandymų metodiką. Suformavę 9 lapus skrotelėje dvimečiai augalai, valgomosios morkos (*Daucus sativus* (Hoffm.) Röhl.) veislė ‘Garduolės’ ir paprastojo kmyno (*Carum carvi* L.) veislė ‘Gintaras’, 120 parų buvo veikiami skirtingais foto ir termo periodais: 0 val. – 4 °C, 8 val. – 4 °C, 16 val. – 21/17 °C, 8 val. – 4 °C, 16 val. – 21/17 °C. Dviejose aptatose *Apiaceae* šeimos rūšyse stebėti skirtingi išsivystymo tempai ir keliai priklausomai nuo aplinkos sąlygų, o tai apsprendė savitą aprūpinimą sacharoze ir GA<sub>3</sub> kaupimasi apikalinėse meristemose žydėjimo iniciacijos metu bei įtakėjo žiedynstiebio formavimąsi morkose ir kmynuose. Mes nustatėme, kad vernalizacija turi stipresnį teigiamą efektą morkų žydėjimo iniciacijai, o aukšta temperatūra stabdo generatyvinių organų formavimąsi. Žydėjimo iniciacija morkose labiau priklauso nuo temperatūros nei nuo fotoperiodo sąlygų skirtingais ontogenezės tarpsniais. Ilga diena ir vernalizacija kmynuose lėmė beveik pilna žydėjimą, aukšta temperatūra, nepriklausomai nuo fotoperiodo apsprendė dalinį žydėjimą, o trumpa diena ir vernalizacija yra limituojantis kmynų žydėjimą veiksnys.

**Reikšminiai žodžiai:** giberelo rūgštis, fotoperiodas, kmynas, morka, sacharozė, temperatūra, vernalizacija.