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Variation of ABA and carotenoids during flowering initiation in carrots

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ABSTRACT The aim - to investigate the dynamics and interaction of abscisic acid and carotenoids in carrot during different flowering initiation stages. The process of carrot flowering initiation and morphogenesis was studied in a phytotron facility: 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, EXP4 – photoperiod of 8 h and 21/16°C temperature, EXP5 – photoperiod of 16 h and 21/16°C (day/night) temperature. High-performance liquid chromatography (HPLC) with diode array detector was used for separation and determination of phytohormones (cytokinin, gibberellic acid, indol-3-acetic acid, and abscisic acid). Spectrofotometric analysis of total carotenoids quantification was made by 644 nm spectrophotometer. The development of carrots with 9 leaves in rosette in different treatments was not identical. Carrots grown in higher temperatures contained a higher level of carotenes in roots than did carrots grown in lower temperatures. Thought such tendencies were not observed in carrot leaves. During flower initiation stage the increase of ABA level in leaves and the decrease in roots was observed in all treatments. The common tendency of carotenoid content variation was observed under treatment with low temperatures and short day photoperiod. These changes can be connected with plant preparation for flowering and seed formation. To summarize, low positive temperatures and short day photoperiod conditioned the most rapid formation of elements of inflorescence axis and the fast growth of generative organs. Also these conditions determined low ABA levels in carrot leaves. Temperature makes the highest effect on carotenoid synthesis in carrot root although in leaves this effect is suppressed by short day photoperiod.
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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 are 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 (Jordan 1993). The carrot plant flowers only after vernalization, *i.e.*, low temperatures are needed for flower initiation (Kraepiel and Miginiac 1997). 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 induc-

tion mechanisms are not interrelated can proceed at different time and stipulate different effects (Duchovskis et al. 2003). According to P. Duchovskis, there are two periods in flowering induction and evocation. The first period of flowering induction is photo induction (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 and Samuoliene 2004).

Abscisic acid (ABA) is a sesquiterpenoid plant growth regulator has multiple functions and is involved in many physiological and developmental processes such as transpiration, germination, dormancy, and adaptation to environmental stress (Rock and Zeevaart 1991). ABA seems to act as a general inhibitor of growth and metabolism, but these effects vary with tissue and development stage. ABA is present in many

growing regions, and its levels do not seem to be reduced in actively growing buds. Nonetheless, ABA applied to hypocotyls, epicotyls, leaves, and coleoptiles is generally inhibitory to growth (Srivastava 2002). Carotenoids represent a diverse and a widely distributed class of pigments. They are synthesized de novo in all chlorophyll-containing photosynthetic organisms (Goodwin and Britton 1988). In plants, carotenoids are essential for photosynthesis (Demmig-Adams and Adams 1996); serve as the precursor for the biosynthesis of abscisic acid (Rock and Zeevaart 1991; Schwartz et al. 1997); and act as coloring agents. In photosynthetic tissues, carotenoids accumulate in the membranes of chloroplasts (Siefermann-Harms 1987), while in non-photosynthetic tissues they accumulate in chromoplasts (Camara et al. 1995). Chromoplasts frequently derive from fully developed chloroplasts, as seen in tomato (*Lycopersicon esculentum*) fruits. In other instances, chromoplasts can arise from non-photosynthetic plastids, as in carrot (*Daucas carota*) roots (Marano et al. 1993). In all cases, chromoplasts accumulate large amounts of carotenoids in specialized lipoprotein-sequestering structures (Vishnevetsky et al. 1999). In plants, carotenoid biosynthesis is a multifaceted and highly regulated process (Harker and Hirschberg 1998).

The aim of this study was to investigate the dynamics and interaction of abscisic acid and carotenoids in carrot during different flowering initiation stages.

Materials and Methods

Carrot *Daucus carota* L. var. *Garduolė2* was initially grown in vegetative tumbler 54x34x15 cm size placed in a greenhouse (16 h photoperiod and 21/16°C day/night temperature). The peat (pH ~6) was used as the substrate. Carrots with 9 leaves in rosette were moved to 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, 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 1-2 g of fresh tissue per sample (from roots and leaves) 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 Y, 2003.

Analysis of abscisic acid (ABA) was performed using a Shimadzu HPLC model 10A chromatographer (Japan) 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 mm²). Mobile phase gradient of 55% methanol in 1% acetic acid for ABA was used. The wavelengths of 254 nm were set in the DAD detector for ABA detection. The total run time for the separations at a flow rate of 1 mL/min was approximately 10 min.

Spectrofotometric analysis (spectrophotometer Genesis 6, USA) of total carotenoids quantification was made by 644 nm spectrophotometer. 0.2g of fresh weight (from roots and leaves) was grounded with $CaCO_3$ and extracted in 100% acetone, according to Vetsthtein (Gavrilenko 1975).

The following chemicals were used: acetone(POCH, Poland), $CaCO_3$ (LaChema, Czech Republic), isopropanol (POCH, Poland), imidazole, ABA (Sigma-Aldrich, Germany), NH_2 -columns (Supelco, USA), methanol and hexane (LaChema, Czech Republic), acetic acid (BOH, England).

Results

The highest concentration of ABA was in carrot leaves before flowering induction and in roots it was very low (Fig. 1A and B). Whenever, such strong variation in carotenoids content was not observed (Fig. 2A and B).

Under treatment with photoperiod of 0 h and 4°C temperatures, the development of carrots stopped after evocation stage II. The concentration of ABA was low and almost equal in both leaves and roots during evocation period I and II (Fig. 1A and B). During evocation period I and II the concentration of total carotenoids was the same in leaves as before flowering induction, and decreased twice in carrot roots (Fig. 2A and B).

The development of carrots with 9 leaves in rosette in dif-

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

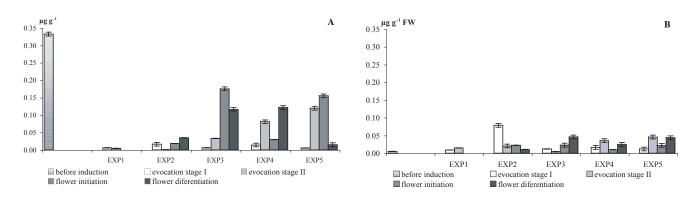| Treatment		EXP1	EXP2	EXP3	EXP4	EXP5
Flowering initiation stage	Evocation stage I (organogenesis stage III)	+	+ + +	+ + +	+ +	+ +
	Evocation stage II (organogenesis stage IV)	+	+ + +	+ + +	+ +	+ +
	Flower initiation (organogenesis stage Vᵃ)	-	+ + +	+ + +	+ +	+ +
	Flower differentiation (organogenesis stage Vᵇ, Vᶜ)	-	+ + +	+ + +	+ +	+ +

"+/-" - development intensity level in carrot.

Figure 1. ABA content variation in carrot leaves (A) and roots (B) during various flowering initiation stages.
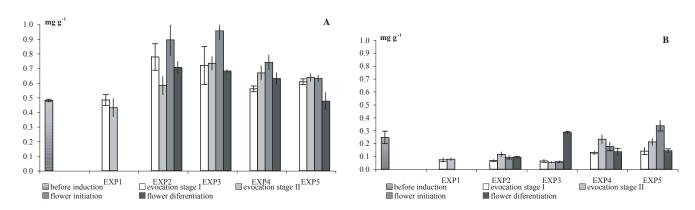

Figure 2. Carotenoids content variation in carrot leaves (A) and roots (B) during various flowering initiation stages.

ferent treatments was not identical. Elements of inflorescence axis were formed (organogenesis stage IV) and the growth of generative organs was the best in treatments EXP2 and EXP3 (Table 1). After flowering induction in evocation stage I, higher contents of ABA were accumulated in carrot roots than in leaves. But in both roots and leaves ABA content was lower (about 2.5 times in leaves) under treatment with long day (LD) than under treatment with short day (SD) photoperiod independently of temperature regime (Fig. 1A and B). And during this stage the highest concentration of ABA in carrot roots and leaves was detected under treatment with SD photoperiod and low temperatures (EXP2) which dramatically decreased during evocation stage II. It was observed the decrease of ABA concentrations in carrot roots during evocation stage II, flower initiation and differentiation processes. The lowest concentrations of ABA were detected in carrot leaves under SD and low temperature treatment (EXP2). The decrease in ABA content also is observed under treatment with high temperatures in flower initiation stage (EXP4) and in flower differentiation stage (EXP5; Fig. 1A).

Whenever, carotenoids concentrations were higher in leaves than in roots in all treatments during various flowering initiation stages. In carrot roots higher concentrations of

carotenoids were detected under treatment with high (EXP4, EXP5) than with low positive temperatures (EXP2, EXP3) treatment during all flowering initiation stages (Fig. 2B). In leaves under treatment with low positive temperature (EXP2, EXP3) the total content of carotenoids increased during flower initiation stage, whereas under treatment with high temperature it was more stable (EXP4, EXP5; Fig. 2A).

Discussion

As it is known, different carrot development level is needed for photo and thermo induction (Duchovskis et al. 2003; Duchovskis and Samuoliene 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 evocation stage first which ends with the formation of the inflorescence axis. After that the second evocation stage (thermo induction) starts. At least 8 assimilating leaves are needed in order to react to thermo induction. During these processes the formation of inflorescence axis elements is complete (Duchovskis et al. 2003). The development of the investigated plants was unequal, the best development rate was under treatment with low posi-

tive temperatures and it lightly depended on the duration of photoperiod.

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 our experiment carrots under treatment with low temperatures showed the more rapid development.

The major ecological function of the carrot root is as a reserve of assimilates for the production of a flowering stem after appropriate stimuli (Hole 1996). It is known that carotenoids are not widely distributed in root crops. Alpha and beta carotene accounts for more than 90% of all carotenoids in carrot (Simon and Wolff 1987). The carotenoid composition is enriched by the presence of biosynthetic precursors, because plants are able to synthesize carotenoids de novo. Carotenoids protect photosynthetic organisms against potentially harmful photo oxidative processes and are essential structural components of the photosynthetic antenna reaction center complexes. In plants, some of these compounds are precursors of abscisic acid, phytohormones, that modulates developmental and stress processes (Koornneef 1986). According to the currently accepted hypothesis, ABA form products of the oxidative cleavage of xanthophylls. Therefore, all steps involved C_{40} compounds are common to both carotenoids and ABA, whereas steps after xanthophylls cleavage are specific for ABA biosynthesis.

Nilsson (1987) found a strong positive correlation between carotene content and accumulated day-degrees above 6°C. Carrots grown in higher temperatures contained a higher level of carotenes than did carrots grown in lower temperatures. Also it is known that carotene content increases with the age and size of the root. These data also correlates with our results observed analyzing carotenoids in carrot root (Fig. 2B). Thought such tendencies were not observed in carrot leaves (Fig. 2A). Also it can be related with carotenoids most important function – the protection against harmful oxygen species in photosynthetic tissues. In nonphotosynthetic tissues, in the absence of carotenoids, plants also suffer severe photo oxidative damage, which generally results in the death of the organism.

The regulatory mechanism of ABA biosynthesis is subject to complex regulation during plant development and in response to environmental stresses (Xiong and Zhu 2003). In vegetative tissues, ABA levels increase when plants encounter adverse environmental conditions (low temperatures). Studies of abscisic acid biosynthesis suggested that ABA in higher plants is synthesized from an "indirect" pathway through the cleavage of a C_{40} carotenoid precursor, followed by a two-step conversion of the intermediate xanthoxin to ABA via ABA-aldehyde (Seo and Koshiba 2002; Schwartz et al. 2003). It was found that carotenoid-deficient plants are ABA-deficient (Quarrie and Lister 1984). The highest level of both carotenoid and ABA was detected in leaves under treatment with low positive temperatures and long day photoperiod during flower initiation stage (Fig. 1A and Fig. 2A). During this stage the increase of ABA level in leaves and the decrease in roots was observed in all treatments (Fig. 1A, B). The common tendency of carotenoid content variation was observed under treatment with low temperatures and short day photoperiod (Fig. 2A). These changes can be connected with plant preparation for flowering and seed formation.

To summarize, low positive temperatures and short day photoperiod conditioned the most rapid formation of elements of inflorescence axis (organogenesis stage IV) and the fast growth of generative organs. Also these conditions determined low ABA levels in carrot leaves. Temperature makes the highest effect on carotenoid synthesis in carrot root although in leaves this effect is suppressed by short day photoperiod.

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