BIOLOGIJA. 2008. Vol. 54. No. 4. P. 279–282
DOI: 10.2478/v10054-008-0057-x

Control of anthesis and formation of reproductive organs in spring rape (*Brassica napus* L.)

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The main goal of this research was to study the process of the formation of reproductive organs in spring rape: flower primordia development, flower formation, their anatomical and morphological structures up to seed formation, as well as the possibility to modify these processes under the effect of the auxin physiological analogues – calcium 4-(2-chloroethoxycarbonylmethy)-1-naphthalenesulfonate (TA-12) and ω-trialkylammonioalkyl ester of 1-naphthylethanoic acid (TA-14).

The anatomical and cytological analysis of vegetative cone in TA-12 and TA-14 treated plants at the stage of 4th–5th leaves showed that compounds stimulated shoot apical meristem (SAM) cell division: the mitotic index (MI) of meristem cells increased by 45% and 63%, respectively, in comparison with the control. For the first time, the results of shoot vegetative cone apical meristem cell division enable to assume that the study compounds initiated oilseed rape vegetative cone development, activated formation of separate flower organs, early seed-bud setting and accelerated the formation of siliquae and seeds.

Key words: spring rape, TA-12, TA-14, reproductive structures

INTRODUCTION

In recent years, particular attention has been paid to realization of the genetically determined productivity potential of oilseed rape, because rape seeds are used not only for food, also but in the production of ecologically pure biological fuel. The low oilseed rape yield in Lithuania can be explained by the lack of theoretical knowledge of oilseed rape growth, reproductive organ formation, absence of modern oilseed rape growing technology applicable in Lithuanian climatic conditions. Investigation of modification possibilities of plant growth, reproductive organ formation under altered phytohormonal conditions are related to the problem of the induction and realization of functional and structural determinants of ontogenesis [1, 2]. Numerous experiments were carried out to optimize genetic information as well to balance the course of ontogenetic processes in vitro and *in vivo* [3, 4].

The formation of reproductive organs is determined by realization of the genetic program; nevertheless, flower and seed initiation and development can be regulated through environmental and endogenous signals. By the exogenous signals, auxin and their physiological analogues control several fundamental aspects of plant growth and development, such as cell expansion, cell division, embryo formation, root development, apical dominance, etc. [5, 6]. The processes of growth and development can be controlled in the desired direction not only with respect to the whole plant, but also to its separate organs as well by applying certain compounds which, not being phytohormones, can act as their physiological analogues, such as TA-12 and TA-14 [2].

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Our earlier investigations of spring oilseed rape var. 'Star' apical meristem development processes and vegetative organ formation have shown that auxin physiological analogues TA-12 and TA-14 are able to induce vegetative cone transition from the vegetative to the generative development stage [4, 7]. This is the reason why the auxin physiological analogues TA-12 and TA-14 are very convenient for studying the peculiarities of oilseed rape flower and seed development. On the other hand, these compounds – plants growth regulators – play an important role in crop production [8].

The main task of this research was to study the processes of anthesis and reproductive organ formation in spring rape: flower primordia development, flower anatomical and morphological structures up to seed formation, as well as the possibility to modify these processes through the effect of auxin physiological analogues – compounds TA-12 and TA-14.

MATERIALS AND METHODS

Spring oilseed rape (*Brassica napus* L. ssp. *oleifera annua* Metzg.) var. 'Mascot' was used as the study object. We studied the effect of auxin physiological analogues TA-12 and TA-14 on mitotic activity of vegetative cone apical meristem cells, flowers, siliquae and seed formation [9]. Small-field trials were carried out at the Experimental Bases of the Institute of Botany. Soil for the trial was prepared by a common technology [10]. The test compounds TA-12 (2 mM) and TA-14 (4 mM) were sprayed at the 2–3 true leaf unfolded stage at optimal concentrations [11] of water solution, 100 ml for each plot. The plot area was 4 m^{-2}. Experiments were performed in four replications.

To estimate the mitotic activity in oilseed rape vegetative cone apical meristem cells, vegetative cones at the stage of 4–5 true leaves were fixed in ice–acetic acid–ethanol mixture (1 : 3). After 4 days, the cones were washed and stained with acetocarmine, whereas cell walls were macerated with chloral hydrate. In temporary squash, the preparations were analysed with a light microscope and a digital video camera (Olympus) (DP-11), the cell mitosis phases were counted and the mitotic index (MI) was calculated. MI is the cell number in mitosis per 1,000 cells of the analysed object in promilles ‰ MI = (M/N) × 1,000, where M is the number of mitoses and N is the cell number [12]. For each variant, 20 vegetative cone apical meristems were analysed.

For anatomical and morphological investigations, the vegetative cones were excised from 20 plants of each variant at the stages of 6–7 and 7–8 true leaves. The prepared samples were fixed in a formalin–acetic acid–ethanol (1 : 1 : 20) mixture, dehydrated in a graded ethanol series, embedded in paraffin and cut with a rotary microtome into 10–15 µm sections [13]. Serial longitudinal sections were stained with Schiff's reagent and analysed with a light microscope and a digital video camera (Olympus) (DP-11). The images were analysed using the Sigma ScanPro 4 (*Jandel Scientific Software*) software.

The obtained data were treated statistically, using the standard Excel 7 computer program.

RESULTS AND DISCUSSION

To increase the productivity and yield stability by applying the auxin physiological analogues TA-12 and TA-14, investigations of the peculiarities of oilseed rape organogenesis, reproductive organ formation and possibilities to control these processes are needed [14]. Anthesis, the flowering duration and the development intensity of reproductive organs are very important for rape productivity formation [4]. The plant development occurs postembryonically, through the reiterative production of organ primordia on the shoot apical meristmen [15]. The SAM initiates the development of primordia and is controlled by environmental and endogenous signals [8].

A cytological analysis of vegetative cones in TA-12 and TA-14 treated plants at the stage of 4th–5th leaves showed that these compounds stimulated apical meristem cell division: in treated plants, the MI of apical meristem cells increased by 45% and 63%, respectively, in comparison with the control.

Cross-sections of the oilseed rape vegetative cones of test variants revealed that meristem cells occurred in the prophase, metaphase and some of them even in the anaphase, whereas most cells of the vegetative cone of control plants were in the prophase (Fig. 1). Rather similar results were obtained in the ex-

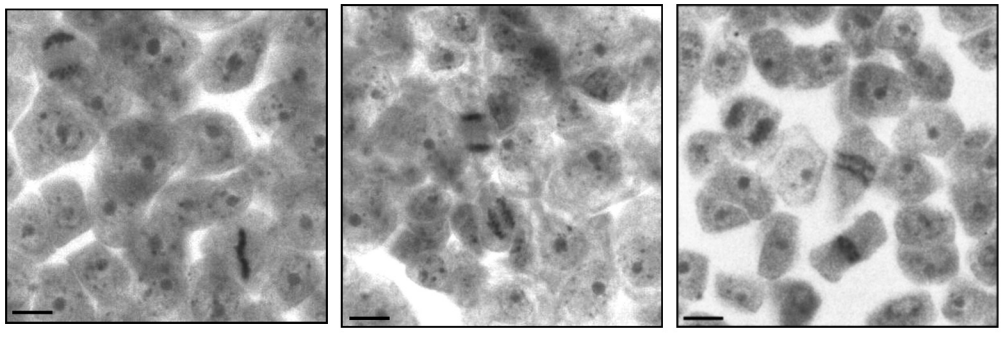

Fig. 1. The effect of compounds TA-12 (2 mM) and TA-14 (4 mM) on the mitotic activity of oilseed rape 'Mascot' vegetative cone apical meristem cells. Bar 20 µm

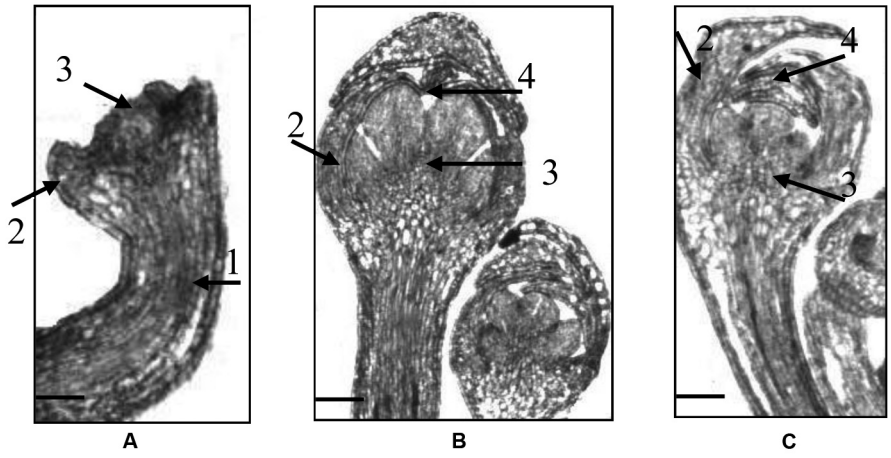

Fig. 2. Effect of the TA-12 (2 mM) and TA-14 (4 mM) on oilseed rape 'Mascot' flower formation at the 6th–7th true leaves stage. **A** – control: pedicle (1), sepals (2), stamens primordia (3); **B –** under the effect of TA-12; **C** – TA-14: pedicle (1), sepals (2), stamens (3) and petals (4). Bar 50 µm

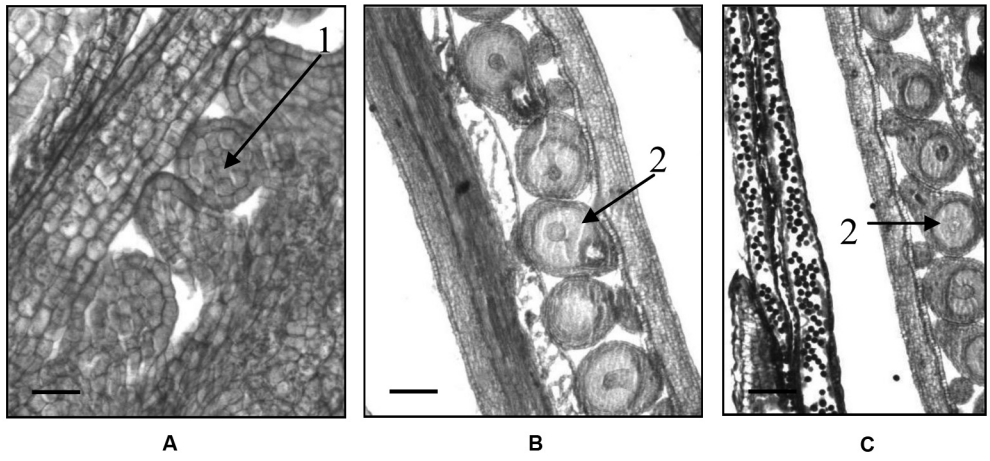

Fig. 3. Effect of TA-12 (2 mM) and TA-14 (4 mM) on the spring oilseed rape 'Mascot' seed initiation at the stage of 7th–8th true leaves. **A** – control: seed-bud setting in the ovary (1); **B –** under the effect of TA-12; **C** – TA-14: fully developed seedbuds in the ovary (2). Bar 50 μm

periments on rape root apical meristem mitosis under the effect of TA-12 [16].

The anatomical analysis of oilseed rape at the stage of 6th–7th true leaves showed that in the control and in the test variants the apices were in the generative stage of development. Plants exposed to the effect of TA-12 and TA-14 had already formed peduncles, petals and carpels, whereas in the control variant only sepal and stamen primordia were formed (Fig. 2).

Investigation in the subsequent stage (7th–8th true leaves) showed that the test compounds, especially TA-14, initiated an early seed-bud setting (Fig. 3). Under the effect of compounds TA-12 and TA-14, an integral process of plant embryo development in the ovule was induced. We observed separate stages of embryo development in the test plants (Fig. 4). The anatomical analysis of developing seeds in the test variants revealed that under the effect of compound TA-12, on average 15% of embryos were in the proembryos stage, 55% in the globule, 20% the heart and 10% the torpedo stages, whereas under the effect of TA-14, most of embryos were in the globule stage (on average 75%), 10% in the heart and 15% in the torpedo stages. At the same time, in the control variant only seed-bud primordia were seen.

For the first time, the obtained data have shown that the auxin physiological analogues TA-12 and TA-14 activated spring oilseed rape shoot apical meristem cell division, induced the initiation of flower primordium, formation of flower anatomical and morphological structures and thus enhanced embryo and seed formation. Model trials with compound TA-12 have shown that this compound can act in plant cells via the system of signal perception and the transduction IAA, a natural auxin, and thus provide for their specific effects [17].

The estimation of the effect of the test compounds on the productivity of intact oilseed rape plants showed that compound TA-12 by stimulating and enhancing the formation of flowers, siliquae and seeds especially on lateral branches, increased the number of siliquae per plant on average by 14% and the seed number by 15% in comparison with the control. Compound TA-14 influenced the formation of productivity elements in the terminal raceme more effectively. The test compounds enhanced the siliquae number per plant by 22% and seed number per siliqua by 17% in comparison with the control (Table).

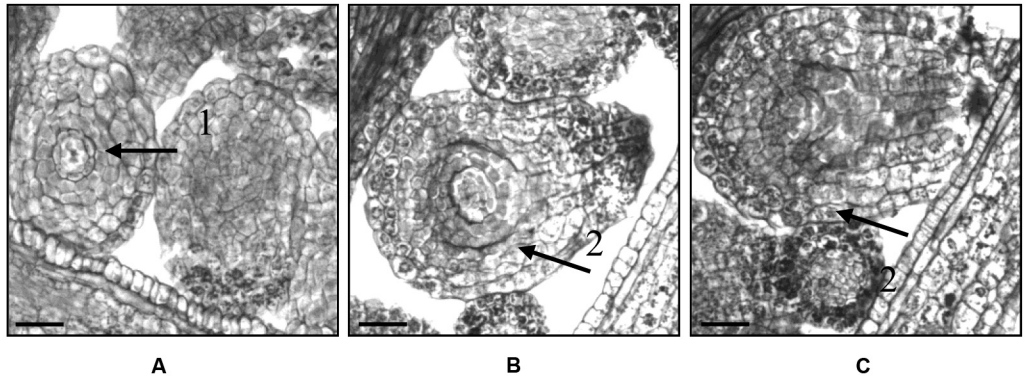

Fig. 4. Effect of TA-12 (2 mM) and TA-14 (4 mM) on the spring oilseed rape 'Mascot' seed formation at the stage of 7th–8th leaves. **A** – control: seed-bud setting in the ovary (1); **B –** under the effect of TA-12; **C** – TA-14: separate stages of embryo development (2). Bar 100 μm

Table. **The effect of TA-12 (2 mM) and TA-14 (4 mM) on siliquae and seed formation in terminal and lateral branches of the oilseed rape 'Mascot' at the stage of full maturation**

Test variant	Number of siliquae per plant				Number of seeds per siliquae			
	Terminal branches		Lateral branches		Terminal branches		Lateral branches	
	n	%	n	%	n	%	n	%
Control (H_20)	22.4 ± 1.7	100	34.4 ± 1.7	100	23.2 ± 1.3	100	20.0 ± 1.5	100
TA-12 (2 mM)	26.6 ± 1.5	119	39.3 ± 1.4	114	26.7 ± 1.3	115	22.9 ± 1.4	114
TA-14 (4 mM)	27.4 ± 1.5	122	40.1 ± 1.5	116	27.3 ± 1.5	117	23.1 ± 1.3	115

The activity of the study compounds depends on related their chemical structure, i. e. on the type of N-substitutients, on the number of methylene groups and presence of trialkylammonio- or chloro-substitutients in the ester fragment [9].

Thus, the obtained results provide new scientific information on the modification of spring rape anthesis process and the development of reproductive organs. The auxin physiological analogues TA-12 and TA-14 could enhance the induction of embryogenesis and formation of productivity elements. Growth regulators, such as auxin physiological analogues TA-12 and TA-14, can be applied to optimize oilseed rape cultivation technologies.

ACKNOWLEDGEMENTS

This work was partly supported by the Lithuanian State Science Foundation (project "Biokuras").

Received 12 April 2008
Accepted 8 September 2008

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VASARINIŲ RAPSŲ (*BRASSICA NAPUS* L.) ŽYDĖJIMO IR REPRODUKTYVINIŲ ORGANŲ FORMAVIMOSI KONTROLĖ

Santrauka

Tyrėme vasarinių rapsų 'Mascot' reproduktyvinių organų formavimosi ypatumus ir auksino fiziologinių analogų – kalcio 4-(2-chloretoksikarbonilmetil)-1-naftalensulfonato –(TA-12 (2 mM)) ir 1-naftiletano rūgšties ω-trialkilamonioalkilesterio druskos (TA-14 (4 mM)) – poveikį šiems procesams.

Anatomine ir citologine rapsų augimo kūgelio analize nustatyta, kad tirtieji junginiai suaktyvino apkalinių meristemų ląstelių dalijimąsi; augalų, paveiktų TA-12 junginiu, mitotinis indeksas (MI) buvo 45%, o TA-14 – 63% didesnis palyginus su kontrole. Anatominiais tyrimais pirmą kartą nustatyta, kad auksino fiziologiniai analogai, skatindami daigo apikalinės meristemos vystymąsi, paankstino atskirų žiedo dalių, sėklapradžio pradmens, ankštarų ir sėklų formavimąsi. Taigi auksino fiziologiniais analogais galima kontroliuoti rapsų vegetatyvinę ir generatyvinę raidą, t. y. su rapsų produktyvumu susijusius procesus. Reikėtų plačiau ištirti auksino fiziologinių analogų panaudojimą optimizuojant rapsų augimo technologijas.