Possibilities to use growth regulators in winter oilseed rape growing technology 2. Effects of auxin analogues on the formation of oilseed rape generative organs and plant winterhardiness

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Absract. The effect of the physiological analogues of auxin, the compounds TA-12 and TA-14, on the apex development, growth, winterhardiness and productivity formation of the winter oilseed rape var. 'Casino' was studied. Field experiments were carried out in 2002–2004 at the experimental station of the Lithuanian Institute of Agriculture, Dotnuva-Akademija. The results showed a positive effect of the compounds TA-12 ($2 \cdot 10^{-3}$ M) and TA-14 ($4 \cdot 10^{-3}$ M) on the autumnal development of oilseed rape plants and their preparation for overwintering: they stimulated the conversion of the apex vegetative phase into a generative one, promoted flower formation and further development, intensified monosaccharide accumulation in the root collum, and improved the winterhardiness of this culture. In test variants, the number of perished plants was lower than in the control. Data of our research demonstrated the functional involvement of sugars in the cold acclimation process of oilseed rape.

Application of compounds TA-12 (417 g ha^{-1}) and TA-14 (369 g ha^{-1}) to plants in autumn at the 4^{th}–5^{th} leaf formation stage not only enhanced winterhardiness but also influenced the further growth in spring and formation of yield components. Under the effect of the compounds TA-12 and TA-14 the number of siliguae on the main and lateral branches racemes and their seed mass increased. All these factors contributed to an extra seed yield by 0.45 and TA-14 by 0.64 t ha^{-1}, respectively, the control yield being 3.53 t ha^{-1}.

Key words: auxin physiological analogues TA-12 and TA-14, apex formation, oilseed rape, winterhardiness

INTRODUCTION

Improvement of different agricultural plant growing techniques as well as the fundamentals of the general theory of plant growth and development should be taken into consideration. It should be also kept in mind that plant species and varieties differ in their specific genetic information concerning growth and development, which is differently used in the course of ontogenesis. The processes of growth and development can be controlled in a desired direction, not only with respect to the whole plant but to its separate organs as well, by applying certain compounds which, not being phytohormones, could act as their physiological analogues (Novickienė, 1995; Darginavičienė & Novickienė, 2002), as shown in experiments with oilseed rape tissue culture and apical meristems of intact spring oilseed rape and *Arabidopsis thaliana* plants (Novickienė & Gavelienė, 2000). It is important to regulate the growth and development of rape plants by active plant growth regulators of the 2-nd and 3-nd generations, which are active at low concentrations, exhibit a specialised selective effect on separate plant organs and are not nutritive substances, leave no residues or metabolites in plant and the environment and are not toxic to humans. It may be physiological analogues of the auxins TA-12 and TA-14 (Novickienė, 1994). The compound TA-12 distinguishes itself by a spectrum of wide effects: it can be applied to activate potato tuber formation and increase sugar-beet root yield and saccharinity. The compound TA-12 is more active in comporison with other compounds: fusicoccine, 6-benzylaminopurine, and epibrassinole (Novickienė & Jurevičius, 1997).

Oilseed rape is the main oil and protein producing plant in Lithuania and one of the main ones in Europe. Oilseed rape seeds are also used in the production of ecologically pure biological fuel. Low seed yields (on average 1.9 t ha^{-1} of spring and winter oilseed rape) in Lithuania can be explained by the lack of theoretical knowledge of oilseed rape growth, generative organ formation, absence of a modern oilseed rape growing technology applicable in Lithuanian climatic conditions. The negative effect of meteorological conditions for oilseed rape wintering is being solved mainly by agrochemical and agrotechnical means such as rational fertilisation of crops, their appropriate density, seed quality, selection of preceding cultures and crop rotations, etc. (Šidlauskas, 1999; Velička, 2002). Winter oilseed rape is nearly twice as productive as spring oilseed rape, however, its winter hardening and overwintering is a challenge.

When solving the problem of how to increase winter oilseed rape endurance, it is necessary to know the factors that predetermine crop survival under unfavourable conditions. It has been noted that under unfavourable wintering conditions winter oilseed rape whose vegetative point is differentiated but not shot up and whose cells are rich in soluble carbohydrates (Gavelienė et al., 1998) displays higher endurance. At the same time, if the autumnal development of a plant slackens, with only 3–4 leaves formed and with the only 3–5 mm thick root collum (Diepenbrock & Grosse, 1995), it cannot accumulate sufficient amounts of sugar and is exposed to a danger of not withstanding the winter frosts (Tumanov, 1979; Morris, 1996). However, there are numerous reports that the role of sugars in increasing freezing tolerance remains unresolved and controversial (Sakai & Larcher, 1987; Uemura & Steponkus, 2003). It is difficult to establish whether the increase in sugar content is causally related to the increase in freezing tolerance or whether it is merely a low temperature response. Plant winterhardiness is induced during the cold acclimation period. It was reported that in this period there changed the expression of separate genes, namely COR 78, COR 15, COR 6.6 (Thomashov, 1999). Nevertheless, cold acclimation is a complex developmental phenomenon and many factors, including protein composition (Anisimovienė et al., 2004; Kasprzewska & Kacperska, 2004) and sugar accumulation, are involved in the increase of cold tolerance.

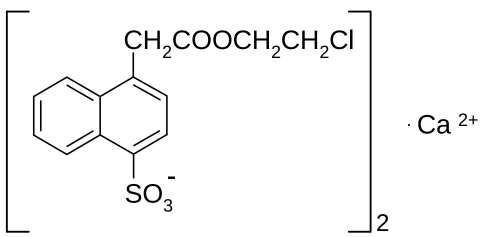
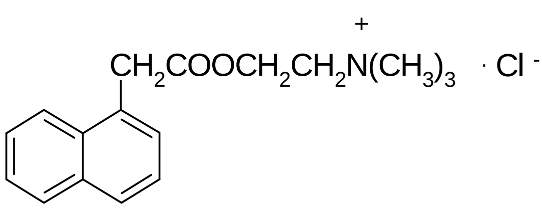
In this work we investigated the effect of physiological analogues of auxin – the compounds TA-12 and TA-14 on winter oilseed rape apex vegetative phase conversion into a generative one, the dynamics of monosaccharide accumulation in root collum in autumn and oilseed rape productivity formation.

MATERIALS AND METHODS

Experiments were performed in small trials with the winter oilseed rape (*Brassica napus* L. ssp. *oleifera biennis* Metzg.) var. 'Casino' in 2002–2004. The plot area under rape was 4.0 m^2 in four replications**.** Plants were treated with water solutions of the compounds TA-12 ($2{\cdot}10^{-3}$ M) and TA-14 ($4{\cdot}10^{-3}$ M) (Table 1) at the 4–5 leaf unfolded stage. Shoot apice samples used in the tests were excised from 10 plants of each variant at the 5–6, 6–7 and 8–9 leaf unfolded stage, (20, 50 and 60 days after the application of the test compounds, respectively). The excised apices were fixed in a formalin – acetic acid – alcohol (1:1:20) mixture and stable histologic preparations were prepared (Kublickienė, 1978). The apices were kept in paraffin, and then cut with a rotary microtome into 10–15 mm thick slices, which were dyed with the Schiff's reagent upon melting the paraffin. These slices were photographed on a light microscope and analysed.

Field experiments with the winter oilseed rape var 'Casino' were carried out in 2002–2004 at the experimental station of the Lithuanian Institute of Agriculture (Dotnuva-Akademija) on a light loamy carbonaceous-gleyic soil containing 2.2% of humus, 250 mg/kg of mobile phosphorus, 225 mg kg^{-1} of mobile potassium, pH 7.0. Before autumn ploughing, the soil was fertilised with $P_{90}K_{60}$ and with N_{90} in spring before the plant vegetation renewal period.

Table 1. Examined compounds.

Structural formula	M.m.	Code
	695,6	TA-12
	307,8	TA-14

The auxin-type analogues TA-12 (417 g ha^{-1}) and TA-14 (369 g ha^{-1}) were sprayed on oilseed rape in optimal concentrations of water solutions, taking 300 l ha^{-1} and 1 l for each plot at the stage of 4–5 true leaves. The concentrations of compounds and the optimum time for their application had been previously estimated by vegetative as well as by field experiments with spring oilseed rape (Novickienė & Gavelienė, 2000). The area of each accountable plot was of 22 m^2, complete in randomised block design, and the experiments were performed in four replications.

The content of monosaccharides in oilseed rape root collum was determined. Samples (20 plants from each variant) were taken in September, October, November, as well as in April. After centrifugation, the monosaccharide content was determined by the orcinic method (Jensen, 1965) and calculated according to a calibration curve, formed on the basis of glucose uptake.

Rape winterhardiness was assessed by counting the number of plants in each plot in autumn and spring, after the overwintering of oilseed rape. To assess the effect of the compounds TA-12 and TA-14 on oilseed rape yield structural elements in the phase of full ripeness, siliquae formation on the main and lateral racemes, their seed mass, and seed number per silique were studied. Upon harvesting, the seed mass from each plot was weighed and the yield was evaluated in t ha^{-1}. The obtained data were statistically evaluated (Songailienė, Ženauskas, 1985). The differences were considered to be statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

After good prewinter development, oilseed rape plants can withstand 0–15°C snowless frost and do not perish even at -20°C under a snow cover. Oilseed rape plants greatly suffer from day and night temperature fluctuations, causing the formation of ice crust and soil distension (Olesen & Grevsen, 1997). Therefore, the autumnal development of oilseed rape leaves may affect not only their wintering and subsequent vegetative renewal in spring, but also the seed yield (Šidlauskas, 1997).
Literature data on the vegetative and generative development regulation in winter oilseed rape are not numerous. More abundant data can be found on flowering induction and initiation in biennial or perennial plants of other classes (Duchovskis, 2000).

An analysis of the anatomy and cytology of oilseed rape apices at the stage of 5th leaf (10 days after the application of the examined compounds) showed that, in the control variant and in variants with the compounds TA-12 and TA-14, the apices were in the vegetative phase (Fig. 1). Differences in the apex development between the control and test variants appeared at the sixth-seventh leaf development stage (50 days after the application). At the eighth-ninth leaf development stage (60 days after application), under the effect of TA-12 and TA-14, floral buds without axillary leaves appeared around the main axis, each giving one floral primordial. In the control, the apex only swelled out and the generative phase of apex became initiated (Fig. 2). Thus, the compounds TA-12 and TA-14 had a considerable effect on the development of winter oilseed rape generative organs and enhanced the process of flowering and productivity elements formation in spring.

Winterhardiness is not a stable property during vegetation: it is formed gradually when the plants are preparing for the winter conditions, i. e. enter a new physiological state. Sugars, proteins and other solutions, irrespective of their chemical origin, can increase frost resistance in cells (Tumanov, 1979, Merkys & Anisimovienė, 1998, Uemura & Steponkus, 2003). Sugars enhance the osmotic pressure in cells and preserve proteins from coagulation by forming with them hydrophilous cytoplasmic compounds. Sugars are particularly protective regarding cell membrane proteins. On the one hand, this results in a higher content of colloid-bound water, which at low temperatures does not freeze and is not transported; on the other hand, the water is retransported into intercellular cavities and, upon thawing, gets back into cells, thus restoring their viability (Tretyekov, 1998). These data served as a basis for the investigation of the effect of the auxin class compounds TA-12 and TA-14 on the monosaccharide accumulation in winter oilseed rape root collum tissues. In the first stage of winter oilseed rape hardening, lasting about 2 weeks, at optimum air temperatures positive (~10°C) in daytime and negative (0–2°C) at night, plants accumulate sugars, proteins, unsaturated fatty acids, etc. (Browse & Xin, 2001). An analysis of monosaccharides in the root collum showed that their accumulation takes part at the first stage of winter oilseed rape hardening. In the subsequent development stages, the content of the saccharides gradually increases and reaches its maximum in November, at the end of the prewintering period (Fig. 3). Results of our research suggest that the examined compounds stimulate monosaccharide accumulation in oilseed rape root collum tissues over the period of plant hardening.

A greater effect on monosaccharide accumulation in the root collum was exercised in the phase of 8^{th} leaf stage (4 November): in test variants the content of these sugars increased by 18% and 15% in comparison with the control (Fig. 3). These results demonstrate the functional involvement of sugars in the cold acclimation of oilseed rape tissues. The enhancing effect of the compounds TA-12 and TA-14 on monosaccharide accumulation in the root collum is related to the fact that the study compounds contributed to the improved winterhardiness. In test variants, the number of perished plants was lower by 11–15% than in the control (Table 2).

On the other hand, the control plants also wintered well, because the meteorological conditions in autumn and winter (except December) in 2002–2003, according to the data of the Dotnuva agrometeorological station, were favourable for the development and wintering of plants. Unfavourable climatic conditions were observed only in December, when the temperature (5.6°C) was lower than the many-year level and the precipitation (18.8 mm) was below the many-year average level (Fig. 4). However, under the effect of the compounds TA-12 and TA-14, the plants showed better hardiness and overwintered well, possibly because of the gradual accumulation of monosaccharides in the root collum of plants.

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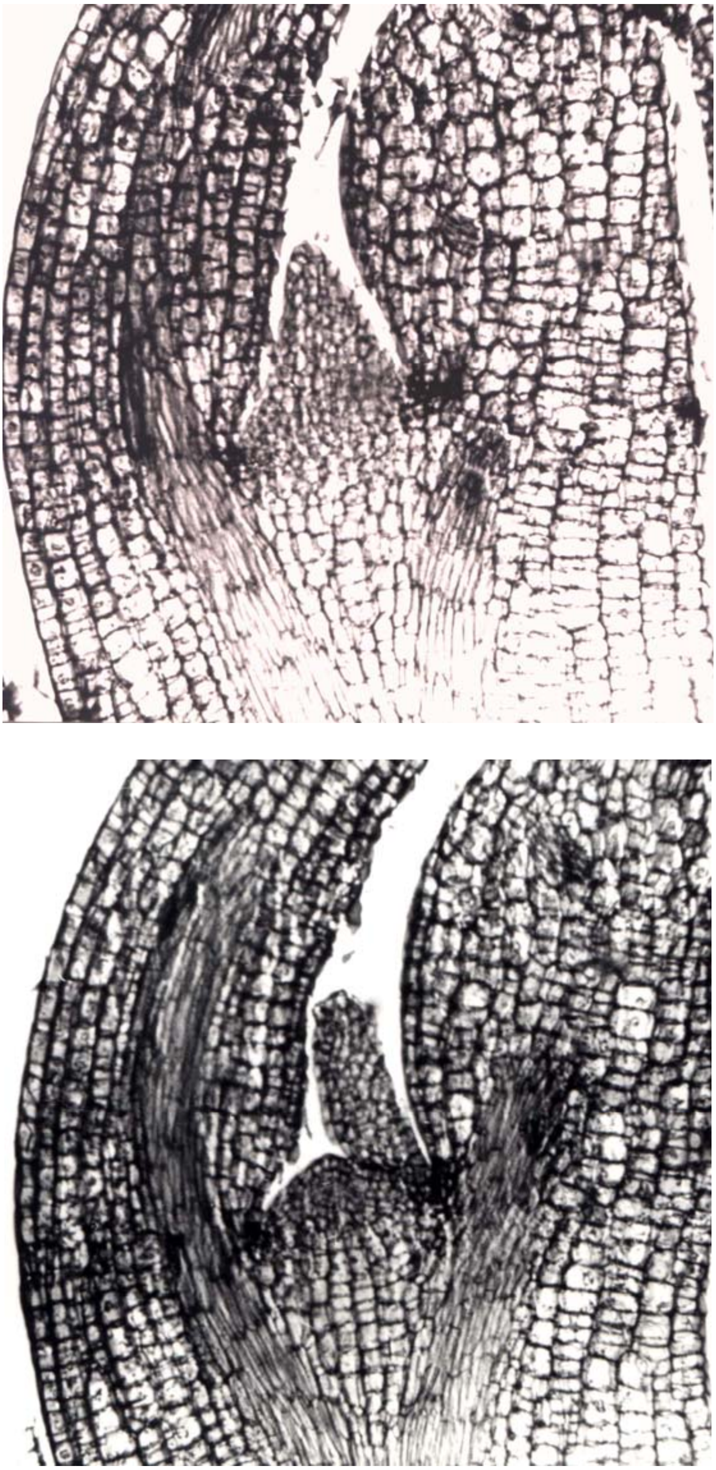


Fig. 1. Effect of the compound TA-12 ($2 \cdot 10^{-3}$ M) on winter oilseed rape apex development (after 10 days application) (×160): 1 – control; 2 – TA-12.

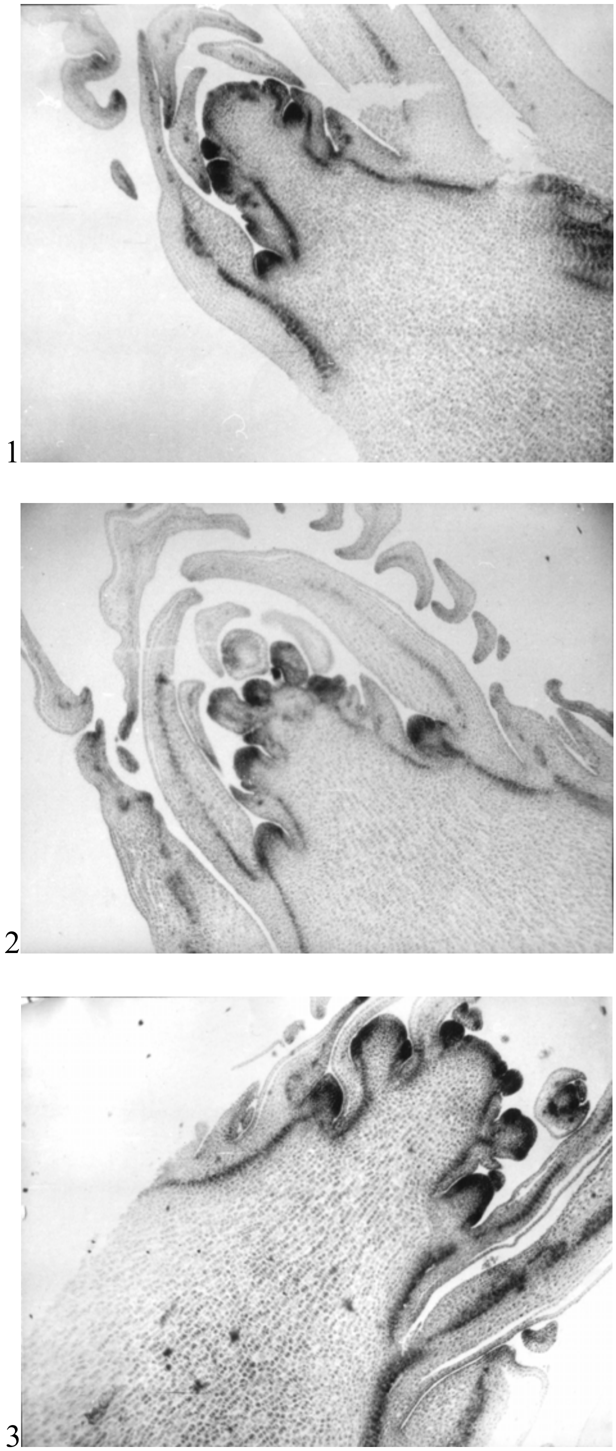


Fig. 2. Effect of the compounds TA-12 ($2 \cdot 10^{-3}$ M) and TA-14 ($4 \cdot 10^{-3}$ M) on winter oilseed rape apex development (after 60 days application) (×160): 1 – control; 2 – TA-12; 3 – TA-14.

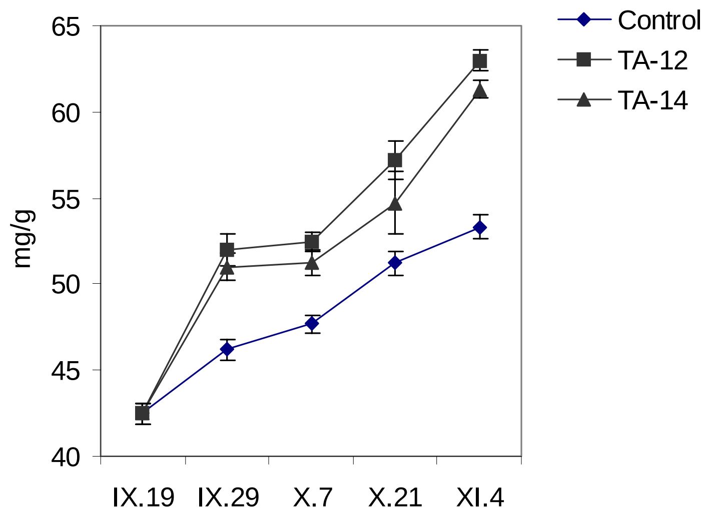
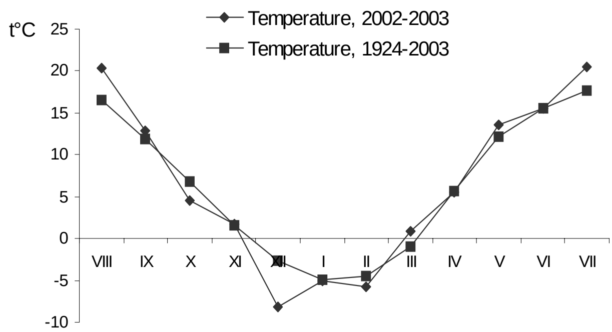


Fig. 3. Effect of the compounds TA-12 ($2{\cdot}10^{-3}$ M) and TA-14 ($4{\cdot}10^{-3}$ M) on the winter oilseed rape ‘Casino‘ monosaccharides content in the root collum.



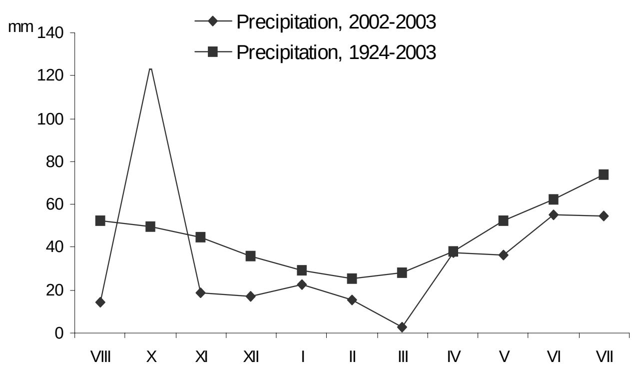


Fig. 4. Meteorological conditions during the winter oilseed rape growing season in 2002–2003 (Dotnuva agrometeorological station).

Table 2. Effect of the compounds TA-12 (417 g ha^{-1}) and TA-14 (369 g ha^{-1}) on the winter oilseed rape 'Casino' plant wintering in 2002–2004.

Test variant	Number of plants, m^2			
	in autumn	in spring	perished number	perished %
Control	86±2,0	60±2,0	27±1	31
TA-12	69±1,0	58±1,0	11±1	16
TA-14	74±1,0	59±1,0	15±2	20

Data are statistically reliable at significance level $P \leq 0.05$.

Table 3. Effect of the compounds TA-12 (417 g ha^{-1}) and TA-14 (369 g ha^{-1}) on siliquae formation and seed yield of the winter oilseed rape 'Casino' in 2002–2004.

Test variant	Number of siliquae per plant						
	main raceme		lateral raceme		total	yield	extra yield
	units	%	units	%	units	t ha^{-1}	
Control	40.5±2.60	100	115.8±1.56	100	156.3	3.53	-
TA-12	48.2±1.50*	119	130.6±3.28	113	178.8*	3.98	0.45
TA-14	50.5±1.86*	125	143.8±4.28*	124	194.3*	4.17	0.64

$LSD_{05} = 0.41$

Note: 40 plants were used per treatment.

*The difference between treated and untreated plants was significant at $P \leq 0.05$.

The examined compounds, positively influencing apex development, enhanced oilseed rape flowering and siliqua formation on the main and lateral racemes. In the phase of ripening, judging from samples taken before harvesting, both TA-12 and TA-14 stimulated the formation of siliquae not only on the main stem raceme but also on the racemes of lateral branches. The total number of siliquae increased by 14% under the effect of TA-12, and by 24% under that of TA-14, compared with the control (Table 3). The seed mass on both on the main stem and lateral branches increased as more siliquae were set and ripened. All these factors contributed to an extra rape seed yield: TA-12 – up to 0.45 t ha^{-1} and TA-14 up to 0.64 t ha^{-1}, the control yield being 3.53 t ha^{-1}.

CONCLUSIONS

1. The physiological analogues of auxin – the compounds TA-12 ($2 \cdot 10^{-3}$ M) and TA-14 ($4 \cdot 10^{-3}$ M), applied on the winter oilseed rape 'Casino' in autumn (at the stage of 4th–5th leaves), stimulated the conversion of the apex vegetative phase into a generative one, exercised a positive effect on monosaccharide accumulation in the root collum and thus improved the winterhardiness of this culture.
2. The compounds TA-12 (417 g ha^{-1}) and TA-14 (369 g ha^{-1}) positively improved the winterhardiness of oilseed rape plants, influencing vegetation renewal in spring and the formation of yield components: more siliguae formed both on the main and lateral branches of racemes and the seed mass there increased as well. All these factors contributed to an extra rape seed yield: TA-12 increased the seed yield by 0.45 and TA-14 by 0.64 t ha^{-1}, the control yield being 3.53 t ha^{-1}.

REFERENCES

Anisimovienė, N., Novickienė, L. & Jankauskienė, J. 2004. Cold acclimation of winter rape: changes in protein composition under the effect of the auxin analogue TA-14. *Žemdirbystė, biomedicinos mokslų srities agronomijos krypties mokslo darbai. Akademija* **86**, 30–38.

Browse, J., Xin, Z 2001. Temperature sensing and cold acclimation. *Curr. Opin. In Plant Biology* **4**, 241–246.

Darginavičienė, J. & Novickienė, L. 2002. Augimo problemos šiuolaikinėje fiziologijoje. Vilnius. LMA. 99.

Diepenbrock, W. & Grosse, F. 1995. Advances in Plant Breeding 17. Supplements of the Journal Plant Breeding. *Beihefte zur Zeitschrift für Pfanzenzüchtung* 21–89.

Duchovskis, P. 2000. Conception of two-phase flowering induction and evocation in wintering plants. *Sodininkystė ir daržininkystė. Mokslo darbai.* Babtai. 19, **(3)-1**, 3–12.

Gavelienė, V., Novickienė, L., Miliuvienė, L. & Brazauskienė, I. 1998. The effect of growth regulators on wheat and rape wintering. *Sodininkystė ir daržininkystė. Mokslo darbai.* Babtai. **17(3),** 195–206.

Jensen, J. 1965. Botaničeskaja gistologija. 173–178.

Kasprzewska, A., Kacperska, A. 2004. Is chitinase class IV involved in Root development in *Arabidopsis* thaliana seedlings? *Acta Physiologiae Plantarum, Special Issue. The 14th Cvongress of the Federation of European Societies of Plant Biology, August 2004,* Cracow, Poland. 69–70.

Kublickienė, O. 1998. *Histologinė technika ir praktinė histochemija.* Vilnius. *Mokslas,* 27–36.

Merkys, A. & Anisimovienė, N. 1998. Frost resistance in plants and methods of its study. *Sodininkystė ir daržininkystė. Mokslo darbai.* Babtai. **17**(3), 29–38.

Morris, DA. 1996. Hormonal Regulation of Source-Sink Relationships:An Over-view of Potential Control Mechanisms. *Photoassimilate Distribution in Plants and Crops. Source-Sink Relationships*. Hong Kong. 441–465.

Novickienė, L. 1994. Physiological principles of modification of ther analogues of phytohormones and retardants, regulating the plant growth, development and productivity. Biology (Plant Physiology) Habil. Dr. Thesis. Vilnius.

Novickienė, L. 1995. Physiological principles of growth and productivity modification of plant organs by applying new analogues of phytohormones. *Biologija.* Vilnius. **3-4**, 18–20.

Novickienė, L. & Jurevičius, J. 1997. The problem of plant growth regulation and prospects of its solution. 1. Physiological analogues of auxin and peculiarities of their activity. *Biologija.* Vilnius. **3**, 61–68.

Novickienė, L. & Gavelienė, V. 2000. Modification of spring rape growth and morphogenesis by physiological analogues of auxin. *Sodininkystė ir daržininkystė. Mokslo darbai.* Babtai. **19(3)-1***,* 180–192.

Songailienė, A., Ženauskas, K. 1985. *Tyrimo duomenų biometrinis vertinimas.* Mokslas, Vilnius.

Olesen, J. E. & Grevesen, K. 1997. Effect of temperature and irradiance on vegetative growth of cauliflower (*Brassica oleracea* L. *botrytis)* and broccoli *(Brassica oleracea* L. *italica). Journal of Experimental Botany* **48**(3/3), 1591–1598.

Sakai, A. & Larcher, W. 1987. *Frost Survival of Plants: Responses and Adaptation to Freezing Stress*. Springer-Verlag, Berlin, Germany. 1–321.

Šidlauskas, G. 1999. The effect of air temperature and agronomic factors on winter oilseed rape leaves area during the vegetative growth in autumn. *Biologija.* Vilnius. **1**, 15–18.

Thomashow, M. F. 1999. Plant cold acclimation: Fresing tolerance genes and regulatory mechanisms. *Ann. Rev. Plant Biol.* **50**, 571–599.

Tretyakov, N. N. 1998. *Fiziologija i biokhimija sel'skokhozeijstvennikh rastenii*. Moskva. 280–286. 440–520.

Tumanov, I. I. 1979. *Fiziologija zakalivanija i morozostoikosti rastenii.* Moskva.

Uemura, M. & Steponkus, L. 2003. Modification of the intracellular sugar content alters the incidence of freeze-induced membrane lesions of protoplasts isolated from *Arabidopsis thaliana* leaves. *Plant, Cell and Environment* **26**, 1083–1096.

Velička R. 2002. *Rapsai.* Latutė, Kaunas.