THE BIOTECHNOLOGICAL TOOLS OF WINTER OILSEED RAPE (*BRASSICA NAPUS* L.) PRODUCTIVITY AND QUALITY CONTROL

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INTRODUCTION

Nowadays oilseed rape is one of the important crops intended to meet food, biofuel and market needs. Rape seed is the main commercially available raw material for biogenic fuel production in Lithuania. However, the cultivation of oilseed rape, especially of winter rape, is rather complicated. In autumn rape is often stressed by snow layer covering unfrozen ground, by very warm and long autumn, by periodic fluctuations of below-zero and above-zero temperatures during snowless winter and early spring, particularly related with climate warming. All these factors are specific to Lithuania (Velička et al., 2005). High quality, double low (00) winter rape varieties developed abroad are cultivated in Lithuania (Novickiene et al., 2009), but they cannot realize productivity potential encoded in the variety genome. Plant varieties differ in their specific genetic information concerning growth, development and seed quality (Novickiene et al., 2009; Amar et al., 2008; Bellostas et al., 2007; Bojanowska, 2006). All should be kept in mind while improving rape growing techniques.

It is known that plant growth regulators play an important role in crops quality and crop production formation (Rademacher and Evans, 1996; Merkys et al., 2007). Using plant growth regulators it is possible to point up potential possibilities of varieties (Rademacher and Evans, 1996; Novickienė et al., 2009).

Rapeseed oil in comparison with other kind of oil contains low levels of saturated fatty acids, large percentage of oleic acid and optimal ratio of poly-unsaturated fatty acids for both nutrition and feeding needs (Butkutė et al., 2000; Hunter, 1990; Mattson, Volpenhein, 1963). The chemical composition of rapeseed, including the fatty acids composition of oil, impacts on the quality of the biodiesel produced (Bellostas et al., 2007; Bojanowska, 2006). Therefore, high importance is ascribed to the rapeseed cultivar, from which rapeseed oil is produced, to foresee its purpose of use.

The main task of this research was to compare the seed mass (yield) and quality of winter oilseed rape varieties *Libea*, *Sunday* and *Valesca* and to study the effect of auxin physiological analogues TA-12 and TA-14 on these parameters of tested rape varieties.

MATERIALS AND METHODS

Winter oilseed rape (*Brassica napus* L. ssp. oleifera biennial Metzg) *cv. Libea*, *Sunday* and *Valesca* were used as research objects. *Libea* (very early variety) was created in Germany, registered in Lithuania in 2002. *Sunday* (medium early) created in Sweden, registered in Lithuania in 2002, and *Valesca*created in Sweden, registered in Lithuania in 1997. The field trials with *cv. Libea* were carried out at the experimental sites of the Lithuanian Institute of Agriculture in Dotnuva-Akademija. The area of each record plot was 22 m^2. The small area field trials with *Sunday* and *Valesca* were carried out at the experimental sites of the Institute of Botany. The plot area was 1m^2. The experiments were replicated four times. Rapeseed was cultivated under local recommendations for fertilisation and oilseed rape plant protection (Brazauskienė et al., 2003). The effect of auxin-type compounds TA-12 (2 mM – 417 g ha^{-1}) and TA-14 (4 mM – 370 g ha^{-1}) on winter oilseed rape productivity elements formation, seed mass, quality, fatty acid composition of oil was studied. The tested compounds were sprayed on oilseed rape plants at the stage of 4-5 leaves (BBCH–14-15) at a tank volume of 300 l water solution per hectare.

Crude fat (CF), crude protein (CP) and glucosinolates (GSL) were analysed by near infrared spectroscopy using NIR Systems model 6500. Equations for the quality prediction were developed at the Lithuanian Institute of Agriculture (Butkutė, 2004).

The extraction of lipids for fatty acids composition was performed by Folch method (Folch et al., 1957). The mixture of fatty acids methyl esters (Christopherson, Glass, 1969) was analysed by gas chromatography GC–2010 SHIMADZU with flame ionization detector.

The data was treated statistically. The presented tables provide the mean values and their standard errors. The significance of difference was assessed by the Student's test; for yield the least significant difference (LSD) was determined at the 95 % confidence level (Tarakanovas, Raudonius, 2003).

RESULTS AND DISCUSSION

At the Laboratory of Plant Physiology (Institute of Botany, Lithuania), it was shown that auxin physiological analogues TA-12 and TA-14 improved oilseed rape development in autumn (leaf formation, increased root collar diameter, monosaccharide content and specific changes in the protein pattern (Gavelienė et al., 2002; Velička et al., 2005; Novickienė et al., 2009). Analysis of winter oilseed rape plants *Libea*, *Sunday* and *Valesca* varieties in autumn 50 days after treatment with tested compounds TA-12 and TA-14 indicated a positive effect on the autumnal development – plant acclimation – preparing for wintering, and vegetation resumption in spring. The number of overwintered plants, having applied TA-12 and TA-14 increased in *cv. Libea* by 11.2 and 9.8 %, in *Sunday* by 8.1 and 7.5%, in *Valesca* by 7.2 and 4.0%, respectively, in comparison with control plants overwintering.

The rape seed yield is the result of a number of complex growth and development processes and growth regulators participation in the following processes. Under the impact of compounds TA-12 and TA-14 siliquae formation on terminal and lateral branches of all tested varieties increased, however the priority was given to siliquae on the terminal branches. Under the effect of TA-14 the number of siliquae in *cv. Libea* increased by 20%, in *Sunday* under the effect of TA-12 – by 23 % in comparison with the control (Table 1).

Table 1. The impact of compounds TA-12 (2 mM) and TA-14 (4 mM) on winter oilseed rape siliquae formation

Test variant	Number of siliquae / plant								
	Terminal branches		Lateral branches					total	
	number	%	I	II	III	IV	V	number	%
Libea Control	44.0±3.8	100	18.9±1.5	20.3±1.9	20.8±1.7	18.5±1.3	16.9±1.4	138.9	100
TA-12	50.5±4.5	115	25.9±2.3	28.2±1.7	28.2±2.3	28.3±2.5	24.6±2.3	185.6	134
TA-14	52.7±4.3	120	25.4±2.4	27.9±2.5	28.9±1.9	27.5±2.1	23.6±1.9	185.6	134
Sunday Control	25.9±3.4	100	10.1±0.9	15.0±1.4	8.2±0.3	5.0±0.3	4.0±0.2	68.2	100
TA-12	44.0±3.9	123	16.0±0.9	14.3±0.5	8.4±0.9	5.2±0.2	4.2±0.3	92.1	135
TA-14	40.1±3.8	112	10.5±0.5	9.2±0.8	7.2±0.5	5.3 ±0.2	4.9±0.3	77.2	113
Valesca Control	36.3±0.8	100	8.9±0.5	8.1±0.4	8.7±0.6	9.3±0.8	7.6±0.8	78.9	100
TA-12	38.7±1.3	107	9.4±1.0	9.2±0.8	10.8±0.9	10.2±0.9	10.0±1.0	88.3	112
TA-14	40.3±1.2	110	11.1±0.6	10.0±0.6	9.8±0.8	9.3±0.7	8.28±0.9	88.8	112

Although analysis of rape seed yield showed, that 1000 seed weight in tested treatments was similar to that of the control, under the impact of compounds TA-12 and TA-14 seed weight of *cv. Libea* increased by 5%, and of *cv. Valesca* by 7 and 10 % respectively in comparison with the control. The 1000 seed weight in *Sunday* cv. of all test treatments was the highest (5.8 g). Under the effect of compounds TA-12 and TA-14 the extra seed yield of *cv. Libea* of 128 and 152 kg ha^{-1}, respectively, was received. In cv. *Sunday* and *Valesca* the statistically reliable yield increment (273 – 137 kg ha^{-1}respectively) was received only under the effect of compound TA-14 (Table 2).

Table 2. The impact of compounds TA-12 (2 mM) and TA-14 (4 mM) on winter rape seed yield

Test variant	1000 seed weight		Yield (8.5 %moisture)		Extra yield	Crude fat yield		Crude fat extra yield
	g	%	kg ha^{-1}	%	kg ha^{-1}	kg ha^{-1}	%	kg ha^{-1}
Libea								
Control	4.25	100	4619	100	-	1986	100	-
TA-12	4.46	105	4747	103	128	2076	104	90
TA-14	4.47	105	4771	103	152	2087	105	100
Sunday								
Control	5.80	100	3191	100	-	1463	100	-
TA-12	5.80	100	3263	102	45	1547	106	84
TA-14	5.78	100	3464	108	273	1648	113	185
Valesca								
Control	4.00	100	3467	100	-	-	-	-
TA-12	4.30	107	3525	101	58	-	-	-
TA-14	4.40	110	3604	103	137	-	-	-
LSD_{05}					115			68

Seed quality analysis by NIRS showed that tested compounds had insignificant impact on fat, protein and glucosinolates content in *cv. Libea* seeds. At the same time the crude fat content was highest in seeds of *cv. Sunday* in control plant seeds 50.1% DM and under the impact of TA-12 and TA-14 crude fat content increased by 3 and 4%, crude protein decreased by 4 and 6%, and glucosinolates content increased by 9 and 10%, respectively, as compared with the control (Table 3). Seeds of *cv. Sunday* accumulate more glucosinolates, than *cv. Libea* seeds. The concentrations of CF under the effect of the tested compounds varied inappreciably within a variety, but obvious difference was found between the varieties *Libea* and *Sunday*.

Table 3. Effects of compounds TA-12 (2 mM) and TA-14 (4 mM) on winter rape seed quality

Variety	Tested variant	Crude fat		Crude protein		Glucosinolates	
		% DM	% from control	% DM	% from control	μmol g^{-1}	% from control

Libea	Control	47.0	100	18.7	100	11.92	100
	TA-12	47.8	102	18.9	101	11.60	97
	TA-14	47.8	102	19.1	102	11.48	96
Sunday	Control	50.1	100	18.2	100	15.31	100
	TA-12	51.8	103	17.5	96	16.71	109
	TA-14	52.0	104	17.1	94	16.83	110

Fat yield was calculated from absolutely dry and pure rape seed yield and CF concentration, and physiologically active compounds application turned out to be significant (at LSD 05) for CF yield (Table 2). Under the effect of TA-12 and TA-14 CF yield in *cv. Sunday* increased by 6 and 13%, respectively, in comparison with the control. The effect of auxin physiological analogue TA-14 on seed and CF yields increase was stronger than that of TA-12 (Table 2).

There are few literature sources on the biochemical rapeseed composition. The sum of crude fat and crude protein in winter rapeseed fluctuated from 64 to 70%. The growing conditions and growing technology can only insignificantly modify this quality parameter (Mendham, 1995; Butkutė et al., 2000). For us a matter of great concern was to determine not only the fat content in different rape varieties, but the fatty acids composition. The content of those mono- and polyunsaturated fatty acids determines, among other things, the viscosity of fuel (Bojanowska, 2006). Monounsaturated acids in rapeseed oil, and oleic acid in particular, are highly valuable for the production of stable fuels, but the presence of polyunsaturated acids in rape-methyl-ester molecules is the cause of their susceptibility to oxidation, which makes them less stable, especially during fuel storage. The obtained results demonstrated different fatty acids composition in tested winter rapeseed varieties (Table 4).

Table 4. Fatty acid composition of oil from different varieties of winter oilseed rape

Fatty acid		*Sunday*	*Libea*	*Valesca*
number of carbon atoms		content in oil, %		
Saturated fatty acids				
C 14:0	Myristic	-	0.27	-
C 16:0	Palmitic	4.65	9.18	4.17
C 18:0	Stearic	1.89	2.29	1.45
C 20:0	Arachidic	0.52	0.45	0.49
C 22:0	Behenic	0.25	0.23	0.25
Total Saturated fatty acids		7.31	12.42	6.36
Monounsaturated fatty acids				
C 16:1	Palmitoleic	0.27	0.36	0.24
C 17:1	Heptadecenoic	0.23	0.15	0.13
C 18:1	Oleic	64.68	61.52	66.26
C 20:1	Eicosenoic	0.91	0.87	0.94
Total monounsaturated fatty acids		66.09	62.39	67.57

Polyunsaturated fatty acids				
C 18:2	Linoleic	18.52	17.52	18.69
C 18:3	Linolenic	8.07	7.16	7.38
Total polyunsaturated fatty acids		26.59	24.68	26.07
		92.68	87.07	93.64
Ratio saturated/unsaturated fatty acids		0.0788	0.1426	0.0679

The content of saturated fatty acids in *Libea* seeds oil was nearly twice as high as that in *Valesca* and *Sunday* seeds oil. This difference is related with increase of palmitic acid content about twice and stearic acid content – by 37 and 17%, respectively. It is noteworthy that *cv. Libea* seed oil contains low levels of monounsaturated fatty acids (oleic) and polyunsaturated fatty acids (linoleic and linolenic) content in comparison with the same fatty acids contents in *Sunday* and *Valesca* seed oil (Table 4). The total amounts of mono- and polyunsaturated fatty acids in oil of rapeseed *Libea*. *Sunday* and *Valesca* are 87.07, 92.68 and 93.64 %, respectively.

First of all our data confirm the opinion that winter oilseed rape quality, including crude fat, glucosinolates and fatty acids composition, depends on the type of variety (Butkutė et al., 2000; Flenet, Merien, 2009). On the other hand, frost and/or cool and wet climatic conditions during the growth season generally lead to high oil content in seeds (Bellostas et al., 2007). The climatic conditions during our experiments (2008-2009) were close to long-time mean, except the winter season of 2008.11- to 2009.03. The temperatures were lower than the long-term mean; through the action of the tested compounds rape plants were well prepared for wintering, exhibited a good over winter survival, resumption of vegetation and formation of productivity elements. Moreover, the temperatures and amount of rainfall in May, June and July were conducive to seed formation and ripening. So the auxin physiological analogues, meteorological conditions, varietal peculiarities have a marked effect on rape seed yield and quality, especially on seed yield of *cv. Libea*, crude fat content in *Sunday* and fatty acids composition in *Sunday* and *Valesca* varieties.

CONCLUSIONS

1. The physiological analogues of auxin-compounds TA-12 and TA-14 applied on winter oilseed rape *Libea*, *Sunday* and *Valesca* plants at 4-5 leaf stage (BBCH-14-15) exerted a positive effect on plant acclimation for wintering, increased over winter survival and affected subsequent plant growth and productivity formation.
2. Compounds TA-12 and TA-14 had a significant effect on siliquae formation and statistically reliable seed yield increasement in *Libea*. Compound TA-14 had a stable effect on seed yield of all tested varieties. Compound TA-14 had

some effect on crude protein content in cv. Sunday and significant effect on fat yield.

3. The varietal genetic peculiarities determined fatty acids composition in rapeseed oil. In cv. *Libea* the content of saturated fatty acids was higher and the content of unsaturated fatty acids was lower, especially that of monounsaturated acids in comparison with fatty acids in cv. *Sunday* and *Valesca*.

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