Tom XXII Rośliny Oleiste 2001

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Effect of sulphur fertilization upon biomass dry matter production dynamics and glucosinolate biosynthesis in three types of winter oilseed rape (*Brassica napus* L.)

Wpływ nawożenia siarkowego na dynamikę produkcji biomasy i biosyntezę glukozynolanów w trzech typach rzepaku ozimego

Słowa kluczowe: rzepak ozimy, linie, mieszaniec, rzepak transgeniczny, nawożenie siarką, plon, pobieranie siarki, biosynteza glukozynolanów

Key words: oilseed rape, lines, hybrid, transgenic OSR, sulphur fertilization, yield, sulphur uptake, glucosinolate biosynthesis

U tří odrůd ozimé řepky: liniové odrůdy Lirajet, její geneticky modifikované formy Lirajet Roundup Ready (GMO) a hybridní odrůdy Pronto, byl v nádobovém pokusu sledován vliv stupňovaného hnojení sírou na dynamiku tvorby sušiny biomasy v průběhu růstu a na výnos semene. Sledována byla též biosynteza glukosinolátů v průběhu růstu za účelem zjištění jejich zastoupení v celkovém obsahu síry ve vegetativních orgánech. Tvorba sušiny v průběhu růstu je ovlivněna typem řepky. Výrazně se odlišuje chování geneticky modifikované formy Lirajet Roundup Ready. Stupňované dávky síry mají positivní vliv na výnos, kdy pro odrůdu Lirajet a Pronto je optimální dávka 80kgS/ha (varianta 3), zatímco pro transgenní odrůdu Lirajet GMO je dostačující dávka 40 kg S/ha (varianta 2) Výnos semene je u liniové odrůdy Lirajet o 4,2%vyšší oproti nulové variantě, u hybridní odrůdy je to pak o 8-9% a u Lirajetu GMO dokonce o 17% vyšší. K výnosu výše uvedených typů řepek je u geneticky modifikovaného Lirajetu dostačující dávka 40 kg S/ha (varianta 2). V příjmu síry je nejvýznamnější list, kde se kumuluje nejvíce síry a proto je také významným indikátorem jejího nedostatku. Příjem síry je ovlivněn typem řepky, kdy geneticky modifikovaná odrůda Lirajet GMO ve fázi listové růžice kumuluje nejvíce síry po ní následuje hybridní odrůda Pronto a nejméně pak liniová odrůda Lirajet. Glukosinoláty jako sekundární metabolity, jsou minoritní složkou sirných sloučenin. Tvoří méně než 2% celkové síry na počátku vegetace a v průběhu růstu, kdy jejich obsah klesá, tvoří méně než 0,1%. Nejvyšší obsahy glukosinolátů má odrůda Pronto.

W pracy badano wpływ wzrastających dawek siarki na dynamikę produkcji suchej biomasy w czasie wegetacji oraz na plon nasion różnych typów rzepaku. Badano trzy typy rzepaku ozimego: odmianę Lirajet, jej generatywnie zmodyfikowaną formę odporną na Roundup (GMO) i odmianę mieszańcową Pronto w doświadczeniu

Three varieties of oilseed rape (OSR) — Lirajet line variety, its genetically modified form of Lirajet Roundup Ready (GMO), and Pronto hybrid varieties were used in a pot experiment to study the effect of increased fertilization with sulphur, the dynamics of biomass dry matter production in the course of growth and the effect wazonowym. Badano również biosyntezę glukozynolanów w czasie wegetacji w celu określenia ich udziału w całkowitej ilości siarki zawartej w organach wegetatywnych. Produkcja suchej masy w czasie wegetacji zależy od typu rzepaku. Odmiana Lirajet GMO różni się istotnie od innych. Wzrastające dawki siarki wpływały pozytywnie na wzrost plonu. Dla odmian Lirajet i Pronto dawka optymalna wynosiła 80 kg S/ha, natomiast transgeniczna odmiana Lirajet GMO zadowalała się dawką 40 kg S/ha. Plon nasion odmiany liniowej Lirajet był o 4,2% wyższy od obiektu bez nawożenia siarką, podczas gdy odmiana mieszańcowa wykazała wzrost o 8–9% a Lirajet GMO nawet o 17%. Stwierdzono, że liście odgrywają najważniejszą rolę, ponieważ gromadzą większość pobranej siarki, są więc bardzo ważnym wskaźnikiem jej braku. Pobieranie siarki jest również zależne od typu rzepaku. Zmodyfikowany genetycznie Lirajet GMO gromadzi większość siarki w stadium rozety, podobnie jak odmiana Pronto i Lirajet. Glukozynolany, będące wtórnymi metabolitami, stanowią mniejszy udział wśród związków siarki. Dają one mniej niż 2% siarki ogólnej na początku wegetacji, a w czasie wzrostu ich zawartość obniża się do poziomu poniżej 0,1%. Odmiana Pronto zawiera największą ilość glukozynolanów.

upon the seed yield. Glucosinolate biosynthesis during the growth phase was also studied with the aim to determine their participation in the total content of sulphur in vegetative organs. Dry matter production during the growth phase is affected by the OSR type. The Lirajet Roundup Ready genetically modified OSR differs significantly from the others. The increasing doses of sulphur affect the yields positively, where Lirajet and Pronto varieties optimum rate is 80 kg S /ha while the transgenic Lirajet GMO is satisfied with 40 kg S/ha. The seed yield in the line variety Lirajet is 4.2% higher than the zero variant while the hybrid variety showed 8–9% and Lirajet GMO had even 17% higher results. The leaf plays the most important role in the S-uptake, as it accumulates most of the sulphur taken, and consequently it is an important indicator of its deficiency. Sulphur uptake is also affected by the OSR type when the genetically modified variety Lirajet GMO in the phase of a leaf rosette accumulates most sulphur, followed by the hybrid Pronto and the line Lirajet. Glucosinolates as secondary metabolites are a minor component of sulphur compounds. They make less than 2% of total sulphur at the start of vegetation and during the growth when their contents fall to less than 0.1%. The Pronto variety contains the largest amount of glucosinolates.

Introduction

Plants generally take nitrogen (N) and sulphur (S) in proportional amounts used to produce proteins (Friedrich, Shrader 1978). The excess or the deficiency of one or both of these elements disturb their synthesis. Interaction observed between N and S showed that N taken in the form of NO_3^{-1}, and S in the form SO_4^{-2} closed metabolic chain which cannot be solved independently (Fismes et al. 1999).

Brassica sp. are characteristic of high S-requirements. Beside its need in the production of S-aminoacids and for the glucosinolate biosynthesis, the increased S-requirement remains an unproven explanation. They should act as a reserve supply of sulphur to be used in its deficiency (Schnug, Hanelaus 1993). Nevertheless, scientific literature lacks such hypotheses since glucosinolates are merely a minor sulphur supply in vegetative tissues of single-zero, double-zero, hybrid and transgenic rapes. Glucosinolates represent less than 5% of total sulphur in OSR leaves and stems (Griffiths et al. 1994) and thus a consideration concerning their importance as an S — supply seems to be unjustified. Their importance is more a defence against harmful factors which is well documented by a complex indolylmetabolism (Helmlinger et al. 1983). A number of studies showed the important role of these glucosinolates in relation to fungous diseases, and they are in line with a hypothesis (Kutáček et al. 1968) that glucobrassicin which is easily transported but hard to cleave is a substance capable of releasing auxin at the time of the plant emergency (frost, disease).

The objective of the present work was a study of the production of vegetative and generative organs of OSR at three S — levels in a pot experiment with three different OSR types: the line variety Lirajet, its genetically modified form Lirajet GMO and the hybrid variety Pronto. The S-uptake through individual organs during the growth phase, and the glucosinolate biosynthesis in all three types, is a part of this work.

Material and methods

The pot experiment

OSR seedling pre-cultivation: sowing date 3.09.98, pots at 10 cm in diameter, filled with 440 g of soil (brown soil — Ruzyně), varieties — Lirajet, Pronto and Lirajet GMO Roundup Ready. Open cold hotbed set — 2.11.98.

Pot experiment set — in proper

9–10.3.99 — seedlings transplanted into vegetation pots filled with 4.5 kg of soil (brown soil — Ruzyně). The soil nutritive state was analysed by diagnostic soil test KEC–UF and total content of C, N and S were determined using the LECO CNS–2000 analyzer.

The soil characteristics data: $pH_{(0,2\ M\ KCl)}$ = 6.7; $C_{(LECO)}$ = 1.215%; $N_{(LECO)}$ = 0.112%; $S_{(total\ LECO)}$ = 112.7 mg S/kg; CEC[*]–UF soil test (Matula, Pirkl 1988; Matula 1996): CEC = 211 mmol(+)/kg, $S_{(ICP)}$ = 20 mg/kg, K = 194 mg/kg; Mg= 72 mg/kg; P = 3 mg/kg; Mn = 3.1 mg/kg. Based on the soil test result the total volume of each pot was mixed with 0.304 g $Ca(H_2PO_4)_2.H_2O$. As the soil was low in magnesium, the increased S — fertilization was provided using magnesium sulphate which was admixed into the soil prior to the pot filling. The $MgSO_4.7\ H_2O$ doses were applied as follows:

variant 1 — 0 kg S/ha, 0 kg Mg/ha
variant 2 — 0.514 g/pot — 40 kg S/ha, 30,4 kg Mg/ha
variant 3 — 1.028 g/pot — 80 kg S/ha, 60,8 kg Mg/ha
variant 4 — 2.056 g/pot — 160 kg S/ha, 121,6 kg Mg/ha.

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[*] CEC — creation exchange capacity,
S_{ICP} — inductively coupled plasma atomic emission spectroscopy (ICP – AES spectrometr)

In the course of vegetation, 0,8 g N was applied per pot in the form of an NH_4NO_3 solution in eight divided doses within the period between 11.03.1999 and 26.05.1999. Each variant was set up in 14 replications.

Sampling scheme during vegetation

- 1st sampling 7.4.1999, phase — end of the leaf rosette formation, indicates stem elongation; plant partition: green biomass and roots;
- 2nd sampling 22.4.1999, phase — buttonisation ends; plant partition: leaves and buds, stem and roots;
- 3rd sampling 13.5.1999, phase — full flowering (main inflorescence 1/3 – 1/2 flowering completed; plant partition: inflorescence, stem, leaves, roots;
- 4th sampling 3.6.1999, phase — ripening (flowering completed, young pods, leaves shedding); plant partition: pod, stem, stem with flower and roots;
- 5th sampling 7.7.1999, phase — harvest; plant partition: pods, seed, stem, inflorescence stem, roots.

Note: the plant samples (except for the last sampling) were frozen immediately using liquid nitrogen and then lyophilised.

Plant part analysis

- HNO_3 mineralisation in a Milestone MLS — 1200 MEGA microwave digesting apparatus: K, Mg, Ca, Mn, S of plant, B and Na, on **ICP — AES** (inductively coupled plasma atomic emission spectroscopy) in the Trace SCAN spectroscope (by Thermo Jareel Ash);
- H_2SO_4, N and P mineralisation;
- $N–NO_3$ and $S–SO_4$ water leach, SAN Plus System analyzer (by SKALAR)
- Glucosinolates: gluconapin (GLN), glucobrasicanapin (GLBN), progoitrin (Prog), Glubr and Neoglubr, as silylderivates desulphoglucosinolates by heat programmed gas chromatography using Hewlett Packard HP 5890.
- Statistical method.

Results were analysed with two — way ANOVA.

Results and discussion

The development of the biomass dry matter at variant 1, 2, 3 and 4 is very similar in all three OSR types up to the beginning of flowering (tab. 1). A significant difference is evident with the sulphur transport from leaves into the reserve organs. The sulphur transport requires a sulphate form and this is why organic sulphur changes into sulphate, which in turn changes back into organic in the reserve organs where it is stored.

The S dose of variant 3 has a significant effect upon the root weight as well as upon the biomass of Lirajet variety at ripening time. The seed yield at this rate is also 4.2% higher (fig. 1) than the other doses. This is proven also by an external experiment (Zukalová et al. 1999). Extreme S-dose (variant 4) is ineffective.

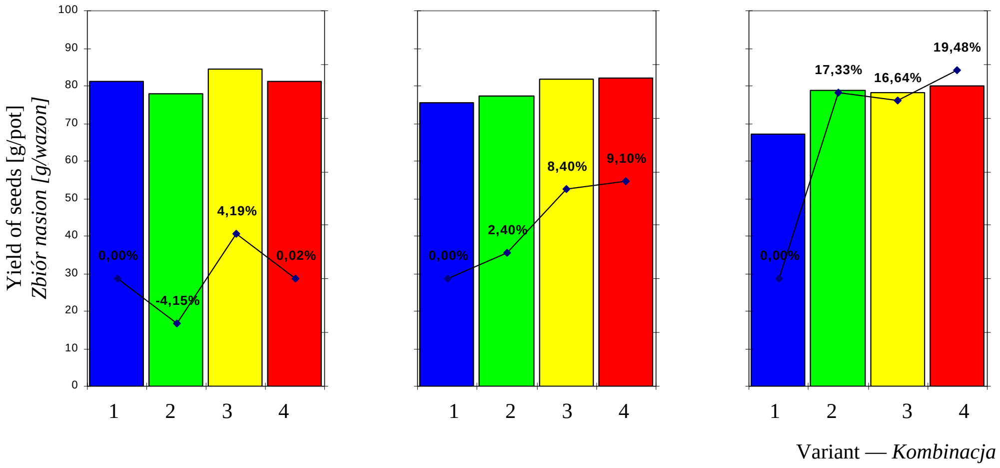
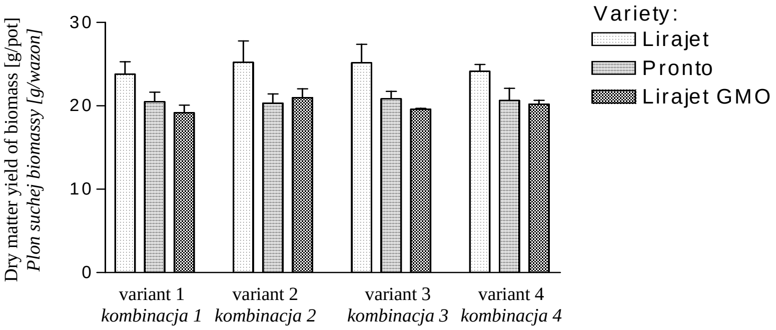


Fig. 1. Seed yields of three winter oilseed rape varieties according to sulphur fertilization
Plon nasion trzech typów odmian rzepaku ozimego w zależności od nawożenia siarką

The rate of 80 kg S/ha (variant 3) has a significant effect upon the increased dry matter weight at the full flowering and ripening time both in the hybrid variety Pronto and the line variety Lirajet. The Pronto variety produces more green biomass dry matter than Lirajet at this rate of S-fertilization. The seed yield is growing progressively with the S-doses (fig. 1, tab. 1) by 8–9% as compared with the 0 kg S/ha dose (variant 1).

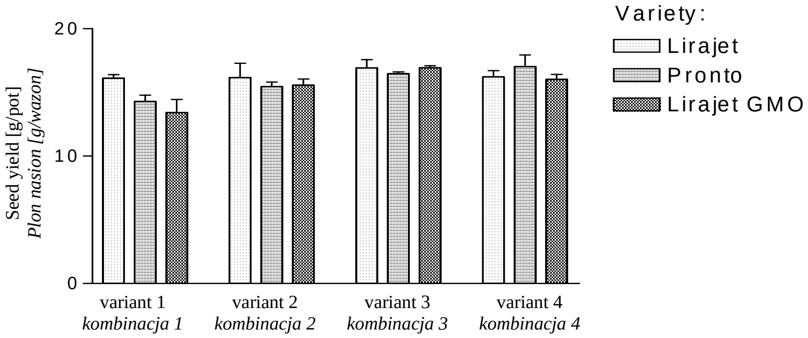
The Lirajet GMO transgenic OSR biomass dry matter behaves quite differently from its line form as well as from the Pronto variety. It produces most biomass at variant 2. The transgenic OSR production capacity is satisfied with 40 kg S/ha (variant 2) when more than a 17% increase of seed yield occurs, and any further application of sulphur is ineffective. This genetically modified OSR form is very sensitive to S-fertilization. The seed yield in transgenic OSR as compared with the line or hybrid type is the lowest at 0 kg S/ha (variant 1). Increasing doses of sulphur balance this difference. The yield is 17–19% higher against the variant 1 (tab. 1).

The genetic basis, i.e. the variety, is of importance for the biomass production, as the statistics show (fig. 2). The effect of the increasing rate of S-fertilization was not statistically significant. On the contrary, the increasing doses of sulphur had a significant effect upon the yield (fig. 3).



Variation *Zmienność*	Degrees of freedom *Stopnie swobody*	Sum of square *Suma kwadratów*	P value	F	Significance *Istotność*
Among varieties *Między odmianami*	2	200,4	< 0,0001	13,63	yes
Among variants *Między kombinacjami*	3	6,519	0,8282	0,2957	no
Error — *Błąd*	0				

Fig. 2. Dry matter yield of biomasse [g/pot] of three winter oilseed rape varieties according to sulphur fertilization — *Zbiór suchej biomasy trzech odmian rzepaku ozimego w zależności od nawożenia siarką*



Variation *Zmienność*	Degrees of freedom *Stopnie swobody*	Sum of square *Suma kwadratów*	P value	F	Significance *Istotność*
Among varieties *Między odmianami*	2	6,245	0,1570	1,950	no
Among variants *Między kombinacjami*	3	32,98	0,0009	6,864	yes
Error — *Błąd*	0				

Fig. 3. Seed yield [g/pot] of three winter oilseed rape varieties according to sulphur fertilization *Zbiór nasion trzech odmian rzepaku ozimego w zależności od nawożenia siarką*

Concentration of total sulphur in separate organs and growth phases for individual OSR types are given in fig. 4, 5, 6. Leaves are the greatest sulphur consumers thus being also an important visual indicator of its deficiency.

The Lirajet line variety and Pronto behave similarly in the S-uptake at increasing S rates (fig. 4, 5). Pronto in its early growth phases is characteristic of a higher total S-uptake against Lirajet. Transgenic Lirajet GMO (fig. 6) takes more S in these early stages than the other two types, but it refuses the extreme S-dose (variant 4) which remains in the soil unused.

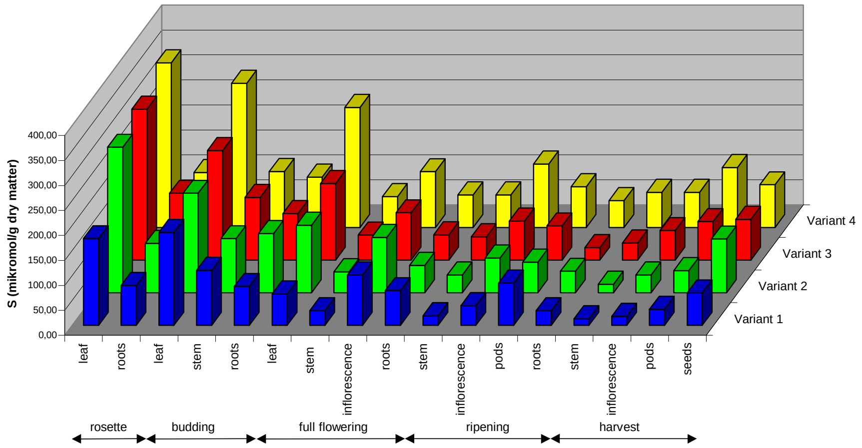


Fig. 4. Content of sulphur in winter rape during growing and seed ripening – variety Lirajet
Zawartość siarki w rzepaku ozimym podczas wzrostu i dojrzewania nasion – odmiana Lirajet

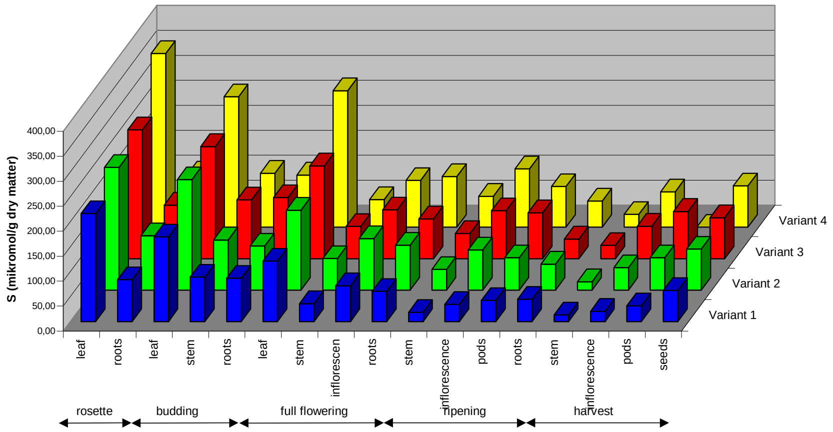


Fig. 5. Content of sulphur in winter rape during growing and seed ripening – variety Pronto
Zawartość siarki w rzepaku ozimym podczas wzrostu i dojrzewania nasion – odmiana Pronto

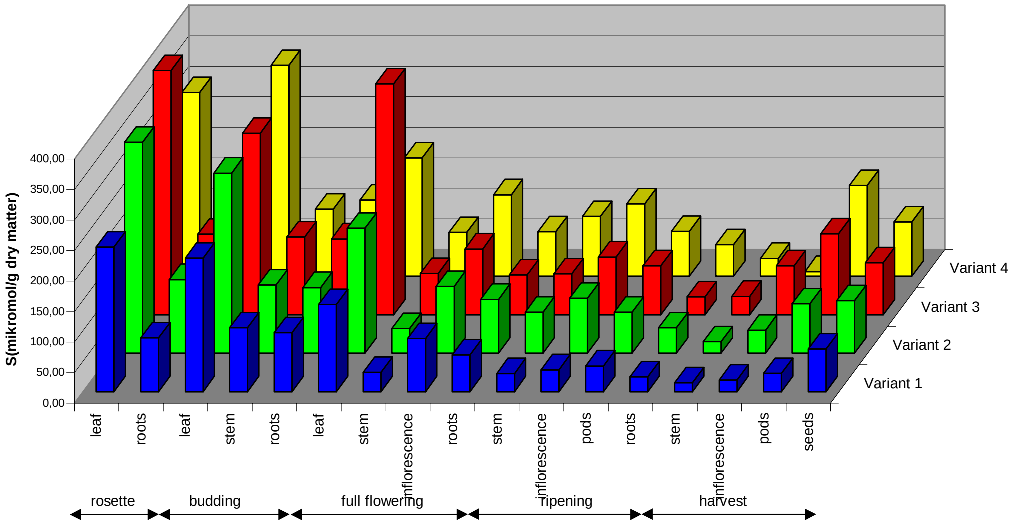


Fig. 6. Content of sulphur in winter rape during growing and seed ripening – variety Lirajet GMO
Zawartość siarki w rzepaku ozimym podczas wzrostu i dojrzewania nasion – odmiana Lirajet GMO

In old leaves sulphur accumulates mostly in the form of SO_4^- while at the beginning of vegetation there is more protein — type S and consequently also glutathion (Zhao et al. 1999) and glucosinolates in the young leaves rather than in the old ones (fig. 4, 5, 6). Glucosinolates in the green matter contain less than 2% of the total S — contents in these OSR types and this share falls further down to 0.1% during the growth, as our results show (fig. 7 and fig. 4, 5, 6). With respect to this comparison the S-uptake was expressed in μmol/g of dry matter against the plant nutritionist tradition of expressing in g/pot. This is in harmony with our earlier results (Zukalová et al. 1991, Porter et al. 1991). It becomes evident that the glucosinolates as minor sulphur compounds cannot affect the sulphur management and they cannot explain the high need of sulphur for the growth and development. Other results and high presence of sulphur in leaves indicate that this is the limiting point in sulphur management.

Table 1

Dry matter production of vegetative and generative organs depending on increasing S fertilization doses (in g per pot)

Produkcja suchej masy wegetatywnych i generatywnych organów w zależności od nawożenia siarką (g/wazon)

Variant *Kombinacja*		Phase — *Faza*								
		rosette — *rozeta*		budding — *pąkowanie*		full flowering — *pełnia kwitnienia*		maturity — *dojrzałość*		harvest *zbiór*
		część nadziemna	roots *korzenie*	*część nadziemna*	roots *korzenie*	*część nadziemna*	roots *korzenie*	*część nadziemna*	roots *korzenie*	
Lirajet	1	7,29	2,64	16,06	8,50	132,46	19,90	151,65	14,15	119,32
	2	6,21	2,31	17,60	5,60	86,13	9,73	97,61	11,10	123,07
	3	5,52	1,97	17,83	5,50	59,31	9,80	161,95	16,75	118,64
	4	6,65	2,31	17,56	6,48	60,13	8,43	149,95	13,95	120,74
Pronto	1	6,69	2,55	22,38	7,75	54,76	17,20	162,95	17,20	104,98
	2	6,69	2,43	21,83	8,03	59,98	12,23	144,95	17,70	101,58
	3	7,67	2,55	21,18	6,30	103,95	15,30	173,95	19,75	105,35
	4	7,03	2,38	19,03	7,13	63,62	11,15	96,37	13,10	100,73
Lirajet GMO	1	5,23	2,36	15,35	5,62	58,18	9,65	176,45	14,55	95,85
	2	5,84	2,33	19,45	6,35	60,04	9,23	117,10	13,17	102,83
	3	5,58	2,48	17,25	4,95	41,48	6,85	82,93	12,10	97,20
	4	6,18	2,20	20,68	7,30	90,05	14,40	164,75	13,40	101,06

Analysis of variance — *Analiza wariancji*

Variation *Zmienność*	Overground — *Część nadziemna*				Roots — *Korzenie*			
	Df	Mean square	F Ratio	P value	Df	Mean square	F Ratio	P value
Among varieties — *Między odmianami*	2	462,379	1,25	0,2944	2	33,4309	7,21	0,0018
Among phases — *Między fazami*	4	38962,5	105,61	0,0000	4	355,268	76,67	0,0000
Among variants — *Między kombinacjami*	3	297,596	0,81	0,4961	3	9,73017	2,10	0,1120
Residual — *Całkowita*	50	368,917			50	4,6338		

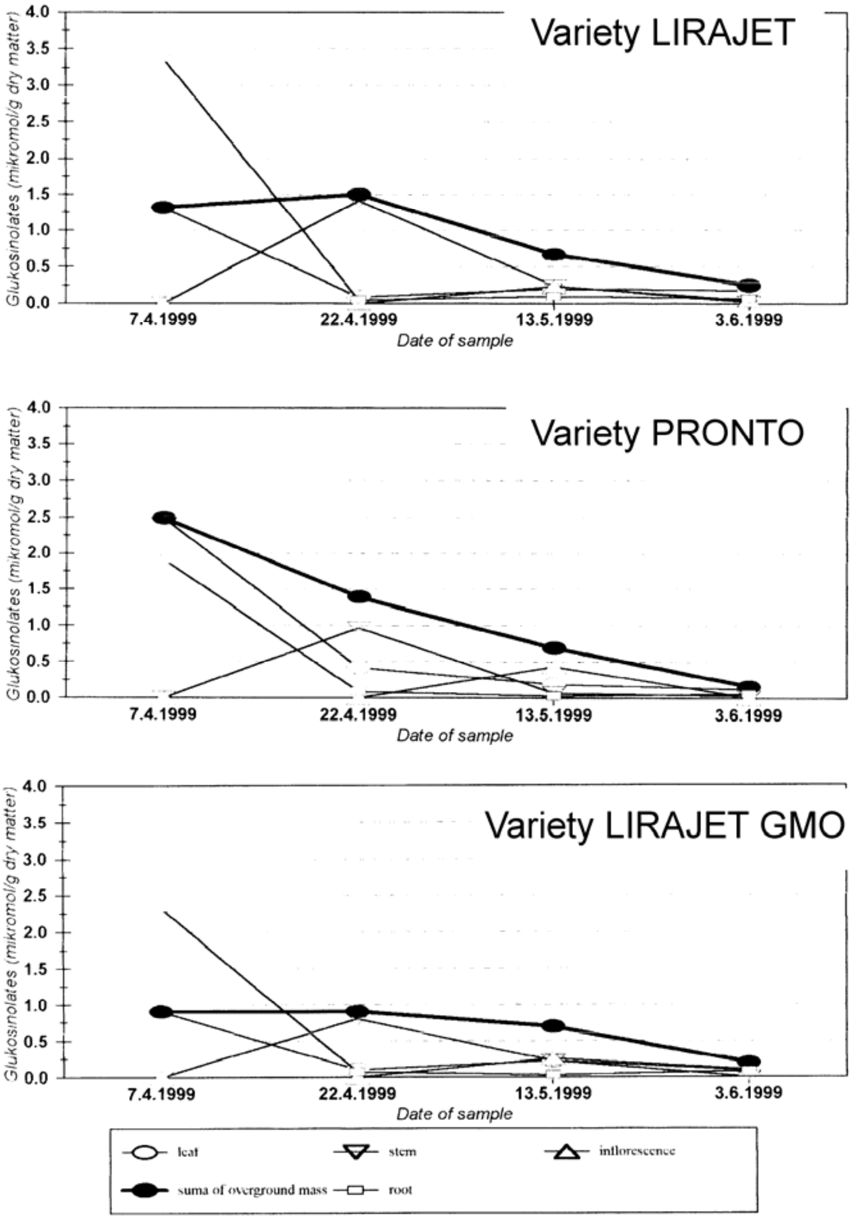


Fig. 7. Changes of glucosinolates content during growth of winter oilseed rape varieties Lirajet, Pronto, Lirajet GMO — *Zmiany zawartości glukozynolanów w rzepaku odmiany Lirajet, Pronto, Lirajet GMO w czasie wegetacji*

Conclusion

1. Production of vegetative organs as depending upon the increasing rates of sulphur is affected by OSR type. Transgenic Lirajet GMO differs significantly producing less biomass at 0 kg S/ha (variant 1) and most at 40 kg S (variant 2) while the line Lirajet and hybrid Pronto do so at 80 kg S/ha (variant 3).
2. Seed yield is affected significantly by increasing rates of sulphur. Just as with biomass, Lirajet GMO differs very significantly from the other two types with decreasing seed yield significantly by lack of S fertilization (variant 1).
3. 80 kg S/ha (variant 3) is the optimal dose of sulphur for the line Lirajet and Pronto as far as the seed yield and SO_4^- accumulation decrease are concerned, while the transgenic Lirajet GMO is satisfied with 40 kg S/ha (variant 2).
4. Glucosinolates are minor components of sulphur compounds. They make less than 2% of total sulphur at the beginning of vegetation and this share falls down to 0.1% in the course of growth. Glucosinolates are highest in Pronto and lowest in Lirajet GMO.
5. The leaf till budding is the most important organ where most sulphur is accumulated and it is an important visual indicator of sulphur insufficiency.
6. Sulphur uptake is also affected by the OSR type, just as the biomass production is, where, again Lirajet GMO accumulates smaller amounts of sulphur in the leaf rosette stage.
7. The preliminary results have already shown that oilseed rape sulphur remains in the vegetative and generative organs in the form of SO_4^- with leaves being the likely point where it is retained.

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Acknowledgements

The autors gratefully acknowledge the support from the Grant Agency of Czech Republic. This work was supported in part from research grant No. 521/99/047 and project EP9233.