Glucosinolates in conventional cultivars and hybrid cultivars of Brassica napus L. with the influence of *pat*-gene and a glufosinate application

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ABSTRACT

Rape plants of an open pollination cultivar (1), a male sterility Lembke (MSL) hybrid cultivar (2), a transgenic herbicide resistant MSL-hybrid cultivar (3) and two transgenic crossings (1 x 3) and (2 x 3) were investigated at the flower stage. The dry matter content did not differ between the cultivars and their transgenic crossings. The transgenic crossings had a significantly higher protein content in dry matter than their parental cultivars. The glucosinolate content of the transgenic crossings was twofold higher than in their parents' cultivars. In an accompanying investigation the glucosinolate content of roots was higher than that of stems and the glucosinolate content of stems was higher than that of leaves. Contrary the protein content decreased from leaves over the stems up to the roots. Testing the herbicide glufosinate at the highest dose resulted in survival only of transgenic plants. Glufosinate tested in a non-toxic dose increased the dry matter content and lowered the glucosinolate and protein content of dry matter.

Key words: indolyl glucosinolates – transgenic – rapeseed – glufosinate

INTRODUCTION

After the successes in the breeding of 00-rapeseed varieties two further steps in the improvement of quality features are being made: (1) breeding hybrids for additional yield, (2) establishing resistance against glufosinate (phosphinothricin, PPT) by fitting PPT acetyl transferase gene (*pat*-gene) into the rapeseed genome.

MATERIALS AND METHODS

In Exp. 1 rapeseed plants of three varieties and crossings were compared: an open pollination cultivar *Lirajet* (1), a male sterility Lembke (MSL) hybrid cultivar *Panther* (2), a transgenic herbicide resistant MSL-hybrid cultivar *Avalon* (3) and two transgenic crossings (1 x 3) and (2 x 3). Each group was represented by 9 plants at the flower stage growing initially under field and then under greenhouse conditions. In Exp. 2 the leaf, stem and root fractions of 5 plants were compared. In Exp. 3 three applications of PPT (without PPT and with a low or a high PPT dosage) on plants from three cultivars in the 9th-12th leaf stage were investigated (5 plants per group). The glucosinolates (GSL) were determined with the official method of the European Commission (1990) by HPLC in the shock-frozen and freeze-dried plants. The dry matter, DM, (drying at 105 °C) and the protein (N, Kjeldahl, x 6.25) were analyzed according to VDLUFA-Methodenbuch III (1994).

RESULTS

Exp. 1: Between the several cultivars and crossings the height varied at the time of the harvest considerably. The average heights from the top of the bloom up to the top of the longest visible root were 0.95 m for *Avalon*, 0.83 m for *Panther*, 0.56 m for *Lirajet*, 0.71 m for *Panther-Avalon-F1* and 0.56 m for *Lirajet-Avalon-F1*. The concentrations of the DM (Tab. 1) did not differ. On the average 134 mg DM per g fresh matter were analyzed.
The plants` protein content in crossings with the transgenic cultivar was significantly higher than in the purely bred cultivars: 324 vs. 238 $mg \cdot g^{-1}$ DM. The protein increase was due to a lower growth of the crossings.

Table 1. Exp. 1 - Dry matter, protein and glucosinolates of above-ground plant parts from *Lirajet, Panther, Avalon* and their crossings[1]

	Dry matter	Protein	Alkenyl glucosinolates	Indolyl glucosinolates	Total gluco-sinolates
	$mg \cdot g^{-1}$	$mg \cdot g^{-1}$	$\mu mol \cdot g^{-1}$	$\mu mol \cdot g^{-1}$	$\mu mol \cdot g^{-1}$
Lirajet	145 ± 26	41^{bc} ± 15	0.7 ± 0.6	0.5^{a} ± 0.4	1.4^{a} ± 1.0
Panther	133 ± 31	30^{ab} ± 9	0.5 ± 0.3	0.2^{a} ± 0.1	0.7^{a} ± 0.5
Avalon	122 ± 5	25^{a} ± 4	0.8 ± 0.4	0.2^{a} ± 0.1	1.0^{a} ± 0.5
Lirajet-Avalon-F1	139 ± 28	49^{c} ± 16	0.6 ± 0.4	1.5^{b} ± 0.7	2.2^{b} ± 0.8
Panther-Avalon-F1	133 ± 18	40^{bc} ± 6	0.7 ± 0.5	1.3^{b} ± 1.1	2.1^{b} ± 1.5

[1] 9 plants per cultivar at flower stage, all values in fresh matter
[a,b,c] Different letters mark significantly different means (Test of Student-Newman-Keuls, P<0.05).

The MSL-hybrids and the plants from conventional cultivars did not differ in their GSL content (approx. 1 $\mu mol \cdot g^{-1}$). However, the F1-crossings showed the twofold concentration of GSL of their parents, particularly an indolyl GSL increase (Glucobrassicin and 4-Methoxy-Glucobrassicin, Fig. 1).

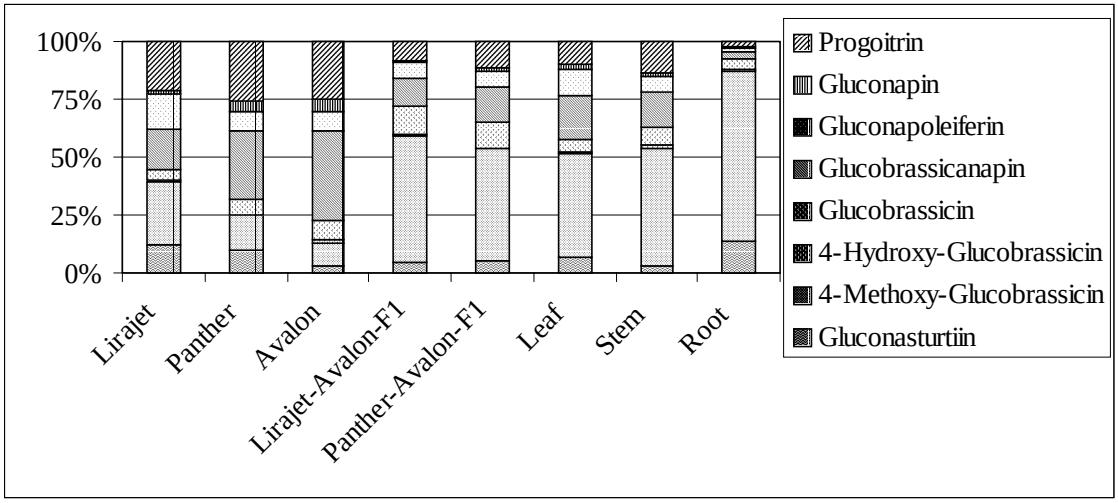

Fig.1. Distribution of glucosinolates in the cultivars of Exp. 1 (above-ground plant parts) and of Exp. 2 (divided into leaf, stem and root)

Exp. 2: The roots contained 4.6 times more DM (Tab. 2) and 35 times more GSL in DM (not shown) than the leaf fraction. In the fresh matter (Tab. 2) the differences of protein and GSL content between the roots and both the investigated above-ground plant parts were lower, but they reached significance, too. The stem GSL content represented the tenfold leaf content and using a nonparametric test the differences were significant. The GSL profile of the roots was dominated by the 4-Methoxy-Glucobrassicin (Fig. 1).
In Exp. 3 with high dosage of PPT only the transgenic cultivar survived (Tab. 3). Administering the lower PPT dose the protein and GSL content in plants` DM was significantly lower in each investigated cultivar, i.e. independent from the occurrence of the *pat*-gene. However, the herbicide treatment led to an increase of DM content by 20 % and therefore the fresh matter of treated and untreated plants showed no group differences in the protein and GSL content.

Table 2. Exp. 2 - Dry matter, protein and glucosinolates in leaf, stem and root – division of a total of five plants*

		Leaf	Stem	Root
Dry matter	$mg \cdot g^{-1}$	$125^{a} \pm 8$	$121^{a} \pm 8$	$577^{b} \pm 50$
Protein	$mg \cdot g^{-1}$	$36^{a} \pm 3$	$28^{a} \pm 5$	$98^{b} \pm 33$
Alkenyl glucosinolates	$\mu mol \cdot g^{-1}$	$0.1^{a} \pm 0.1$	$0.7^{a} \pm 0.4$	$2.4^{b} \pm 1.6$
Indolyl glucosinolates	$\mu mol \cdot g^{-1}$	$0.1^{a} \pm 0.1$	$1.1^{a} \pm 0.9$	$24.1^{b} \pm 25.3$
Total glucosinolates	$\mu mol \cdot g^{-1}$	$0.2^{a} \pm 0.1$	$1.8^{a} \pm 0.7$	$30.8^{b} \pm 31.2$

[a,b] Different letters mark significantly different means (Test of Student-Newman-Keuls, P<0.05). Differences between GSL concentrations of leaf and stem are significant by the U-test according to Mann and Whitney, P<0.05.

* One plant per group of Exp. 1

Table 3. Exp. 3 - Dry matter, protein and glucosinolates of above-ground plant parts from *Falcon, Artus* and *Avalon* after application of glufosinate

	Falcon	*Artus*	*Avalon*
Glufosinate dosage $g \cdot L^{-1}$ water			
	Dry matter [$mg \cdot g^{-1}$ fresh matter]		
0	$97^{a} \pm 7$	$96^{a} \pm 9$	$95^{a} \pm 7$
0.2	$117^{b} \pm 8$	$118^{b} \pm 10$	$125^{b} \pm 6$
1.0	-	-	$117^{b} \pm 7$
	Protein [$mg \cdot g^{-1}$ fresh matter]		
0	23.6 ± 1.9	23.9 ± 1.8	22.9 ± 2.2
0.2	23.4 ± 1.7	22.4 ± 1.4	23.1 ± 1.5
1.0	-	-	22.4 ± 1.3
	Total glucosinolates [$\mu mol \cdot g^{-1}$ fresh matter]		
0	0.08 ± 0.03	0.13 ± 0.05	0.08 ± 0.04
0.2	0.11 ± 0.05	0.08 ± 0.04	0.06 ± 0.02
1.0	-	-	0.06 ± 0.02

[1] 9th to 11th leaf stage

[a,b] Different letters mark significantly different means (Test of Student-Newman-Keuls, P<0.05).

4. DISCUSSION

The insufficient development of the crossing plants in comparison to the plants of parental high-breed sorts agrees with experiences of plant breeders and this shall not be explained here. The higher GSL content of crossings corresponds with their protein-content exceeding the mean contents of the respective parents. There is a correlation in seed protein and GSL content (Hartung et al. in these Proceedings) pointing to links in the protein and GSL synthesis. On the other hand, the crossing plants with their minor stature represented a higher leaf : stem relationship compared to the better developed parent plants. The leaves contained more protein than the stem and the higher protein content of the crossings could be a consequence of the higher leaf and/or of the lower stem part. In case of the GSL the higher leaf- stem relationship of the crossings can not be the reason for their higher GSL content: the leaves contained significantly less GSL than stems (Tab. 2). Therefore, the increased GSL content of the crossing plants cannot be sufficiently explained at present. Using the herbicide below an acute-toxic dose the increased DM – equally in not transgenic and transgenic plants – seems to represent an unspecific response.

REFERENCES

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