Composition of glucosinolates in biomass of brassica genus and their role in crop system

Helena Zukalová[1], Jan Vašák[1], Petr Kroutil[2], David Bečka[1], Vlastimil Mikšík[1]

[1]*Czech Univerzity of Agriculture, Departmentbof Crop Production, Prague 6 – Suchdol, 165 21 Czech Republic*
[2] *State Phytosanitary Administration, Těšnov 17, 117 05 Praque 1, Czech Republic Email: Zukalova@af.czu.cz*

Abstract

Glucosinolates are discussed besides their anti – nutrition effect, also as compounds which create natural defend and with its chemical composition they are ranked among natural pesticides and natural biofumigators. The principle of these effects is passive protection created by the two – component system, glucosinolates – myrosinase. The two – component system of glucosinolate – myrosinase is activated only due to an attack diseases, insects and in the case biofumigators is soil after ploughing in their biomass as green fertilizing or after ploughing in after harvest the left overs of rape. In accordance was observed content and composition of glucosinolates during growth. The most resistance against diseases and insects has white mustard (Sinapis alba) and radish (Raphanus sativus), which differ from Rape, Turnip rape and Brassica juncea, where prevail aliphatic decomposition products. White mustard contains aromatic sinalbin and radish has 4 - ethylsulfinylbutyl glucosinolat – glucoraphanin.

Biofumigative effect of Brassica preceding crops lies on the content and composition of Brassica roots glucosinolates considering that during growth the content of glucosinolates decreases and transported in the generative organs and go over from pods into seeds.Brassica crops (Brassica napus var. napus biennis and napus annua, Brassica campestris, Brassica juncea var. biennis, Sinapis alba, Raphanus sativus var. oleiferus) are more suitable preceding crops for the consequently grown winter wheat than the wheat itself. Winter wheat grown after Brassica preceding crops had less leaves on the main stem invaded by leaf spots, with minor intensity and substantially less number of Septoria leaf blotch on the flag leaf and it also gave higher yields with higher TSW. These Brassica preceding crops positive results are not only caused their natural biofumigators, but also their large capability to improve physical quality of soil and her microbial activity.

Key words: glucosinolates; natural biocides; round-sowing edges; bio-fumigants; *Brassica juncea*; *Sinapis alba*;*Raphanus sativus*;*Brassica campestris*; *Brassica napus* L.; diseases, pests

Introduction

Rapeseed ranks among competitive crops of Czech agriculture and their subtitute on sowing area is on possible abroad.. On the one hand it goes to high pressure of diseases and pests. With this is in connection with extensive using of insecticides and fungicides, which has negative influence on keep balance agroecosystem. Considering that is necessity use of all means to restriction these negative influences and to use natural defensive system, which is one´s own of Brassica family. On the second.hand rape has a stable possition in crop rotation and therefore its influence on the next crop is study. Results of various authors, have one common point- rape belongs to the best forecrop for winter wheat

The aim of this work is the evaluation of glucosinolates with the Brassica genus during growth leading to clarify its role in the plant, evaluation of their natural protection and biofumigation potential leading to purification of the soil without the use of synthetic pesticides.

Material and methods.

The experiment was carried out at the experimental ground of the Research Station of the Faculty of Agronomy of the CUA in Červený Újezd, Prague. Soil - deep brown soil, storage of P, K, Mg good, colloid complex fully saturated, 405m above sea level, average annual temperature 7,9°C and average rainfall 507mm. The content of glucosinolates during growth was followed with:line variety of *Brassica napus.*L variety Navajo (CPB Twyford Ltg.,GB), *Brassica campestris.*L. variety Rex (NPZ H.GLembke KG, Holtsee,D) and Brassica juncea**,** (VNIIMK Krasnodar R a Agrada, CZ, hybrid of summer Brassica juncea and winter rape), *Sinapis alba*. L variety Veronika and *Raphanus sativus* var. oleiferus. The experiment was carried out by standard breeding technology (VAŠÁK and kol., 2000) in form of incidentally chosen plots in 4 repeated forms with an area of 10m2 each.

The creation of biomass was followed in ca 14 day' s intervals by takings of 10 plants and their drying. The average sample of fresh mass was lyofilized and later analysed for the content of different glucosinolates.

Determination of different glucosinolates by the method of gas chromatography.

The content of different glucosinolates was set by gas chromatography after conversion to desulfogluconosinolates and a following silylation of N-methyl-N-trimethylsilylheptafluorobutyramide to silylderivates of glucosinolates according to own modification of the HEANEY method (1986). Silylderivates were then set on the gas chromatograph Hewlett Packard HP 5890 by the method of inner standard. Sinigrin was used as inner standard and for Brassica juncea glucotropaeolin was used.

For statistical evaluation the analysis of dispersion of the programme system STATGRAPHICS was used.

Results and Discussion

• Natural protection of the plant itself

Glucosinolates are listed among the natural pesticides, which are produced by the higher plants for increasing their resistance against the unfavourable effects of the predators, competitors and parasites since they exhibit the toxic or repellent effects and, therefore, they have important position in the protective mechanism of rape plant against pests and diseases (MITHEN, 1992; WALLSGROVE at al., 1999). Two significant classes of the natural pesticides, among them even glucosinolates can be listed, are created by phyto-alexines and phyto-anticipines. The basic difference between the two listed classes is based on the mechanism of their creation:

• *Phyto-alexines* originate as the result of an external influence induced by the modified metabolic activity of the plant *de novo* (they represent the active protective mechanism).

• *Phyto-anticipines* originate from already created precursors, which are produced by a healthy plant from the very beginning of its growth start and that serve only as the passive protection against possible pests. Glucosinolates are the typical example of such precursors. The two-component system of glucosinolates - myrosinase represents the in advance prepared protective system, which is activated only due to an attack and subsequent damage of the plant tissue, after which the enzymatic hydrolysis of glucosinolates occurs with the creation of bio-active iso-thio-cyanates.

This mechanism create principles of:

1. Natural protection of Brassicacea itself
2. Round – sowing edges
3. Bio-fumigatory effects Brassicacea for the subsequent crops- winter wheat.

Iso-thio-cyanates are the significant substance of the synthetic bio-fumigants, where, apart from the aliphatic iso-thio-cyanates (SARWAR et al., 1998), are also aromatic forms that exhibit higher toxicity.

Due to the evaluation of the natural protection of Brassicacea and their biofumigation effects for the following crops we have studied the content and composition of glucosinolates and their decomposition products during growth (Tab.1)

Tab.1: Composition glucosinolates (GSL) in Brassicaceae

Sort	Principal GSL*	Total GSL content (mg/100g biomass)			
		budding	root	ripening	root
Brassica juncea var. biennis	SINI, NAPI	33	12	107	9
Brassica napus L. var.biennis	NAPI, BRNA, PROG, NAPO, GB, NGB, MGB	16 – 45	6 – 14	16 – 21	4 - 11
*Brassica campestris L.*var. biennis	NAPI,BRNA, PROG,	34	28	13	22
Sinapis alba L.	SNB	27	37	197	37
Raphanus sativus var. oleiferus	RAFA, RAFE	23	33	209	21

* *Aliphatic* -SINI-sinigrin, NAPI gluconapin, BRNA glucobrassicanapin,PROG progoitrin, NAPO gluconapoleiferin, RAFA glucorafanin, RAFE glucorafenin, *Cyclic*– SNB sinalbin, *Heterocyclic(Indoly)* -GB glucobrassicin, NGB neoglucobrassicin, MGB 4-hydroxyglucobrassicin

Ad. 1.) White mustard (*Sinapis alba)* and *Raphanus sativus* have to the most important of Brassica diseases- *Phoma lingam* and *Verticillium dahliae,* aret the most resistance (Tab.2), while turnip rape (*Brassica campestris*) was hundred- percent hitted.

Tab. 2: Occurence of diseases Brassicacea forecrop.

Sort	Sclerotinia sclerotiorum (%)	Phoma lingam (%)	Verticillium dahliae.(%)	Alternaria brassicae (scale)[2)]
Brassica napus L. var. *biennis -Navajo*	1	85 (19)[1)]	0	7
Sinapis alba L. *veronika*	0	0	0	9
Brassica campestris L.var. *biennis*. REX	18	100 (100)[1)]	79	7
Raphanus sativus var. *oleiferus*	0	4	0	6
Brassica juncea var. *biennis*	11	60 (13)[1)]	83	5

Note :) [1)] total attack – primary attack; rest is attack in consequence feeding
[2)] Alternaria brassicae – scale attack of surface leaf (9 = dońt attack, 1 =total attack)

Brassica napus L.and Brassica juncea were attacked after these. *Brassica napus* L.– variety Navajo has genetically given resistance to Verticilium.

From these results is clear, that cyclic glucosinolates of Sinapis alba and sulfinic glucosinolate of Raphanus sativus fulfil role of defensive mechanism(Tab.1) in contrast to aliphatic glucosinolates of Brassica napus L., Brassica campestris and Brassica juncea. Besides these mechanism, role plays also genetic code resistance by winter rape variety Navajo, in case Verticilia, which wasńt attacked, while Brassica campestris and Brassica juncea were attacked in high extend (Tab.2) inspite is similar content and composition of glucosinolates.

As regards of pests, released the enzymatic hydrolysis of glucosinolates occurs with the creation of bio active aliphatic isothiocyanate, which have effects as attractante for Melighetes aeneus and Ceutorhynchus assimilis.

Content and composition of glucosinolates and their released products by Sinapis alba and Raphanus sativus have effect as repellent (Tab.3)

Tab.3: Total number of pests.

Sort	*Melighetes aeneus*	Psyll [1]	Phyll [1].	*Ceutorhynchus napi*	*Ceutorhynchus quadridens*	*Ceutorhynchus assimilis*	*Dasyneura brassicae*	Parazitoid
Brassica napus L. var. *biennis -Navajo*	540	0	0	0	2	1688	21	69
Sinapis alba L. *Veronika*	36	0	151	0	0	14	0	0
Brassica campestris L.var. *biennis* REX	272	0	0	0	0	2214	5	22
Raphanus sativus var. *oleiferus*	91	0	59	0	0	15	0	5
Brassica juncea var. *biennis*	755	0	21	8	6	654	26	35

[1]Pozn.: Psyll =Psylliodes chrysocephala, Phyll Phyllotreta

Ad.2 Round – sowing edges-princip is the same as defensive system of plant.Released aliphatic isothiocyanates from glucosinolates of winter turnip rape or spring rape sowing on autumn are considering to large attack pests able to catch the main their pressure and insecticide application can be decreased from three whole –area spring sprays to one whole area application and two sprays on round – sowing edges (tab.4,5).

Tab.4: Number of *Melighetes aeneus* on terminals 100 of plant**.**

		25m	50m	100m
Round – sowing edges - mixture	144	89	42	7
Round – sowing edges - rape	91	87	48	63

Tab.5: Number of *Ceutorhynchus assimilis* on terminàls 100 of plant

		25m	50m	100m
Round – sowing edges - mixture	181	92	21	5
Round – sowing edges - rape	174	107	62	26

Ad. 3. The function of glucosinolates as bio-fumigants.

It is based on the same hydrolytic principle as the natural protection of the plant with the different fact that the ploughed in bio-mass of green manure leaves the bio-active iso-thio-cyanates in soil, which have the significant bio-fumigatory effects (KIRKEGAARD et al., 1999) for the subsequent cultivation of vegetables, particularly. The same effect have even the post-harvest residues of rape plants, which, due to iso-thio-cyanates contained in them, have the significant bio-fumigatory effects for the subsequent crops and, therefore, the rape plant is the unique remedial pre-crop for cereals.

Iso-thio-cyanates are the significant substance of the synthetic bio-fumigants, where, apart from the aliphatic iso-thio-cyanates (SARWAR et al., 1998), are also aromatic forms that exhibit higher toxicity.

Considering that number of attacked leaves of main stalk of winter wheat were marked smaller with smaller intensities. Marked smaller was occurence of *Septoria nodorum* on the flag leaf in compare with forecrop -wheat (Tab.6). Individual *Brassicacea* forecrop behave to occurence of diseases specific (Tab.6). We can common state marked improving at leaf spot and Septoria nodorum on the flag leaf of winter wheat after brassicacea forecrop in compare with winter wheat as forecrop.

Tab. 6: diseases of winter wheat Ebi.

Forecrop	diseases(flag leaves, stalk, ear)							
	leaf spot	Pseudocercosporella herpotrichoides (%)	Septoria nodorum		Puccinia		Erysiphe	Fusarium
	(% surfaceof leaf)		(% surfaceof leaf)	Ears (%)	flag leaves (scale)	ears (%)	flag leaves(% surface of leaf	wheat ears (%)
Brassica campestris Rex	13,2	10	31,3	70	4,8	5	0	13
Brassica rape Pronto	9,8	15	31,3	35	5,5	8	0	28
Brassica rape annua Star	7,4	13	28,8	40	5	0	0	25
Brassica rape Prestol	12,3	0	22,5	63	5,8	0	do 0,25	30
Mixture Rex+Star+Prestol	14,4	0	37,5	43	4,8	0	0	28
Brassica juncea	11,0	0	26,3	75	5,3	3	do 0,25	20
Sinapis alba Veronika	11,4	3	23,8	30	5	3	do 0,25	33
Winter wheat - Ebi	17,4	0	92,5	55	6	0	0	23

Note: was evaluated 10 stalkl, ears and flag leaves from four repetition
Scale 1 - 9 (9 =without Puccinia, 1 = total attack

Conclusion

1.Natural protection of Brassicacea itself - his role fulfil cyclic glucosinolates and their released products of *Sinapis alba* as the same behave sulfinil glucosinolates of *Raphanus sativus* to diseases and pests..

2. Round – sowing edges – aliphatic iso-thio-cyanate- released products of glucosinolate of *Brassica napus* and *Brassica campestris* attack as atractants to Melighetes aeneus *and* Ceutorhynchus assimilis.

This is possibility use for bioprotection by means of round – sowing edges.

3.Biofumigation of Brassicacea – Rape has a stable position in crop rotation Therefore rape belongs to the best forecrop for winter wheat - increasing of yield compared the forecrop cereal was observed and the less occurence of *Septorium nodorum.*

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