Glucosinolate Content in Rapeseed in Relation to Suppression of Subsequent Crop

Satoko Yasumoto1, Morio Matsuzaki1, Hisako Hirokane2 and Kensuke Okada3

1Biomass Production and Processing Research Team, National Agricultural Research Center, 3-1-1, Kannondai, Tsukuba 305-8666, Japan; 2Vegetable and Tea Function Research Team, National Institute of Vegetable and Tea Science, 2769, Kanaya, Shima-dai 428-8501, Japan; 3Research Strategy Office, Japan International Research Center for Agricultural Sciences, 1-1, Ohwashi, Tsukuba 305-8686, Japan

Abstract: In crop rotations that include rapeseed (Brassica napus L.), the growth of the crops following rapeseed is sometimes inhibited. The aim of this study was to assess the role of glucosinolates (GSLs) in the inhibitory effect. Three cultivars with zero erucic acid content (Asakano-natane, Kizakino-natane, Nanashikibu: single-low cultivars) and one cultivar with zero erucic acid and low GSL contents (Kirariboshi: double-low cultivar) were grown. The GSL contents differed greatly depending on plant part, stage of development, and cultivar. Progoitrin and gluconapin were detected mainly in the seeds of the single-low cultivars. Their contents either did not change or increased slightly during the reproductive stage. The double-low cultivar Kirariboshi contained almost none of either progoitrin or gluconapin in any part during the reproductive stage. Glucobrassicanapin, glucobrassicin, and gluconasturtiin were detected, mainly in the roots, of all four cultivars, and tended either to decrease or to remain steady as plants matured. Dense stands of rapeseed seedlings that had germinated from seeds dropped at harvest grew together with the subsequent crop. The GSL contents in the leaves and roots of these seedlings were high. These results suggest that the GSLs in the seeds of single-low cultivars, in the roots of both types at harvest, and in the leaves and roots of volunteer seedlings are the candidate cause of the generally observed phenomenon of inhibited growth of the crop following rapeseed.

Key words: Allelopathy, Gluconasturtiin, Glucosinolates (GSLs), Rapeseed (Brassica napus L.).

Rapeseed (Brassica napus L.) is an important crop for the production of vegetable oil for human consumption, and of biodiesel more recently. In warmer regions, such as the Yangtze River basin in China, in northern India, and in central to western Japan, rapeseed is usually grown as a winter crop in rotation with other summer crops. Rapeseed and other Brassica species have phytotoxic effects on the subsequent crop (Vera et al., 1987). For example, in Japan, the early growth of sunflower and soybean planted immediately after rapeseed was severely suppressed, but not that after barley or winter fallow (Okada et al., 2008).

Cruciferous plants, including rapeseed, contain glucosinolates (GSLs) in their parenchyma (Röbbelen and Thies, 1980). All GSLs are degraded by endogenous myrosinase (thioglucoside glucohydrolase EC3.2.3.1) (Kawagishi, 1985). The major GSLs in rapeseed and their major breakdown products are listed in Table 1. These products inhibited the growth of fungi and oomycetes (Smith and Kirkegaard, 2002) and nematodes (Potter et al., 1998). In addition, volatilization of isothiocyanate (ITC), one of the GSL degradation products from turnip-rape mulch, in soil reduced weed seed germination (Peterson et al., 2001). Both volatile and water-soluble hydrolysis products of GSLs inhibited seed germination (Brown and Morra, 1996). Oleszek (1987) reported that volatiles from B. napus leaves inhibited the germination and the root growth of some weeds and crop plants, but did not study double-low cultivars or other plant parts.

Assuming that GSLs were responsible for the suppression of summer crops after rapeseed, we postulated four hypotheses: (i) The GSLs in the leaves of rapeseed that are usually shed before harvest inhibit the growth of the subsequent crop as the leaves decompose. (ii) The GSLs in the empty pod-shells, stems, and branches cast out by the combine harvesters, and many seeds left from the shattered pods inhibit the growth of the subsequent crop. (iii) GSLs in the rapeseed roots all of which remain in the soil at harvest affect the growth of the subsequent crop as the roots decompose. (iv) The GSLs in the young plant tissues or living plants (voluntary seedlings) that had sprouted from the seeds left after harvest and emerging together with the subsequent crops inhibit the growth of the subsequent crop.
The purpose of this study was to assess the changes in GSL contents in various parts of rapeseed plants at the seedling and reproductive stages and to test the above hypotheses.

**Materials and Methods**

1. **Plant materials**

We grew four rapeseed cultivars, Asakano-natane (Norin No. 46), Kizakino-natane (Norin No. 47), and Nanashikibu (Norin No. 49) which have no erucic acid (single-low cultivars), Kirariboshi (Norin No. 48) which has no erucic acid and only a small amount of GSL in the seed (double-low cultivar).

2. **Field experiments**

Field experiments were conducted in 2004–2005 and 2005–2006 in the Yawara Experimental Field (Tsukubamirai City, Ibaraki, Japan) of the National Agricultural Research Center (Tsukuba City, Ibaraki, Japan). The experiments were conducted in a rotational paddy field in upland...
conditions. Seeds were sown on 13 October 2004 and 27 October 2005, in rows 0.3 m apart, with 5 cm plant spacing in 30-m × 12-m plot. One plot was prepared for one cultivar. Before planting, fertilizer was applied at 7.2 g N m⁻², 6.0 g P₂O₅ m⁻², and 6.0 g K₂O m⁻². Additional ammonium sulfate was top-dressed at the beginning of stem elongation and at flowering at 4.0 g N m⁻² each time. Weeds were removed manually.

An experiment on rapeseed seedlings that had germinated from seeds dropped from disrupted capsules to analyze voluntary seedlings was conducted in July 2006.

3. Sampling

On 25 April (flowering time), 9 and 23 May, and 6 and 20 June (maturing time) 2005, and on 14 and 25 April (flowering time), 23 May, and 20 June (maturing time) 2006, three to four plants were collected. On 8 July 2006, seedlings at the one- to two-leaf stage were collected from 1-m × 1-m quadrat in three replications and we counted the plants in each sampling area. Immediately after sampling, all samples were separated into leaves, pods (further separated into seeds and shells at harvest), stems (hypocotyl in the case of seedlings) and roots. The samples were frozen in liquid nitrogen and then vacuum-freeze-dried (Laytant LFD-600CS2, Laytant, Kanagawa, Japan).

4. Extraction of GSLs and preparation of desulfo-GSLs

GSLs were analyzed as previously described (Ishida et al., 1995; Kim et al., 2004). Freeze-dried samples were ground to a fine powder in a UDY mill (UDY, Tokyo, Japan). A ground sub-sample (200 mg) was then placed in a 10-mL test tube. Crude GSLs were extracted with 5 mL 80% (v/v) boiling methanol in the capped test tube in a water-bath at 70ºC for 30 min during which the endogenous myrosinase was inactivated. The mixture was then centrifuged (21,040 g, 3 min at 4ºC), and the resulting supernatant was collected. The residue was re-extracted twice. The bulked crude extract was applied to a mini-column (using a 1000-μL pipette tip) packed with DEAE-Sephadex A-25 (40 mg dry weight). GSLs were desulfated with 0.5 mL 0.2% aryl sulfatase (15 units). Aqueous sinigrin (allyl glucosinolate) solution (100 μL, 0.2 mg mL⁻¹) was added to the column as an internal standard. After reaction at room temperature overnight, the desulfo-GSLs were eluted three times into a 10-mL test tube with 1 mL distilled water. The extracts were Millipore-

![Fig. 2. Changes in the contents of major glucosinolates in different parts of rapeseed cv. Kiranboshi in 2005. Seed was sown on 13 October 2004. Each value is the mean of three or four replicates and line shows standard error.](image-url)
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5. HPLC analysis

The desulfo-GSLs were separated and detected by the methods of Ishida et al. (1995) and Kim et al. (2004) with slight modification. HPLC was performed with a class-VP chromatograph (Shimadzu, Kyoto, Japan) fitted with an Intersil ODS-3 column (250 mm×4.6 mm i.d., particle size 5 μm; GL Science, Tokyo, Japan). The temperature of the column oven was set at 35ºC. The injected sample volume was 20 μL. GSLs were eluted and separated by linear gradient elution with deionized water and acetonitrile as described (ISO, 1992) at a flow rate of 1.0 mL min⁻¹. The eluates were detected at 228 nm by a UV detector (SPD-10AVvp; Shimadzu, Kyoto, Japan). Three replications were used per sample. Each GSL was identified by comparison with reported chromatograms (Ishida et al., 1995).

6. Statistical analyses

Results are presented as means ± standard error of the mean. Analysis of variance was performed with the statistics program SPSS 11.0J (SPSS Inc., Chicago, IL, USA). The contents of major GSLs in the single-low cultivar were compared with those in the double-low cultivar by t-test at the 5% and 1% levels of significance.

Results

1. Changes in GSL contents in different plant parts during reproductive stage

In the single-low cultivar Kizakino-natane, the content of progoitrin in pods was high, and that in other parts was low and decreased with maturation (Fig. 1). The contents of gluconapin and 4-hydroxy-glucobrassicin in pods increased with maturation; those in other parts were low and decreased with maturation. Contents of glucobrassicanapin, glucobrassicin, and gluconasturtiin were higher in roots than in other parts and decreased with time. The other single-low cultivars showed similar results (data not shown).

In the double-low cultivar Kirariboshi, the contents of progoitrin and gluconapin were almost nil in all parts (Fig. 2), but 4-hydroxyglucobrassicin in pods increased toward harvest and that in other parts were low and decreased with maturation. Contents of glucobrassicanapin, glucobrassicin, and gluconasturtiin were higher in roots than in other parts and decreased with time. The other single-low cultivars showed similar results (data not shown).

2. GSL contents at harvest

At harvest, all leaves had fallen. In the single-low cultivar Kizakino-natane, progoitrin and gluconapin occurred mainly in the seeds. In the double-low cultivar Kirariboshi,
could not be responsible for the inhibition of the growth of the subsequent crop due to GSLs. The GSL content of rapeseed leaves at flowering is much lower than that of stems and roots (Rothe et al., 2004). In *Brassica juncea* L., the GSL content of leaves reportedly declined rapidly following incorporation into soil, and only small quantities remained after 6 d (Gary and Lincoln, 1999). Therefore, little or no GSLs from the fallen leaves remain at the sowing of the next crop.

At harvest, significant amounts of progoitrin and gluconapin were found in pods in the single-low cultivar Kizakino-natane (Fig. 1), mostly in the seeds (Table 2). On the contents were very low (Table 2). Glucobrassicin and gluconasturtiin were present mostly in the roots in both cultivars. At harvest, there were no significant differences (P > 0.05) between single- and double-low cultivars in the contents of these two GSLs in their roots (Table 2, Fig. 3). However, the contents differed with the year (Fig. 3).

3. GSL contents in seedlings

In the field (where the preceding crop was the double-low cultivar Kirariboshi), many rapeseed seedlings germinated in early summer. The content of gluconasturtiin in their roots was markedly high (Fig. 4). The contents of glucobrassicanapin in the leaves and roots were also moderately high. We calculated the amount of each GSL per unit land area (based on an average density of 591 plants m\(^{-2}\)). The highest was glucobrassicanapin in leaves, followed by gluconasturtiin in roots, gluconasturtiin in leaves, and glucobrassicanapin in roots. Total major GSLs content in roots was 298 mmol m\(^{-2}\), which corresponded to 26% of that in the whole plant (Table 3).

### Discussion

To elucidate the cause of the poor growth of crops after rapeseed, we analyzed the changes in the contents of GSLs in different plant parts.

Although content of glucobrassicanapin of leaves was high in the younger plants (Fig. 4), the GSL contents of leaves at flowering were very low or almost nil (Figs. 1, 2). Therefore, the fallen leaves of rapeseed after flowering could not be responsible for the inhibition of the growth of the subsequent crop due to GSLs. The GSL content of rapeseed leaves at flowering is much lower than that of stems and roots (Rothe et al., 2004). In *Brassica juncea* L., the GSL content of leaves reportedly declined rapidly following incorporation into soil, and only small quantities remained after 6 d (Gary and Lincoln, 1999). Therefore, little or no GSLs from the fallen leaves remain at the sowing of the next crop.

At harvest, significant amounts of progoitrin and gluconapin were found in pods in the single-low cultivar Kizakino-natane (Fig. 1), mostly in the seeds (Table 2). On
the other hand, the double-low cultivar Kirariboshi contained only small amounts in pods (Fig. 2) or in seeds and shells (Table 2). Stems of both cultivars contained almost no GSLs, but the roots contained some (Table 2). Thus, GSLs in seeds dropped at harvest of the single-low cultivar or in the roots of both cultivars could affect the growth of the subsequent crop.

The glucobrassicacinapin content of leaves and glucoraphanin content of leaves and roots in the volunteer rapeseed seedlings were very high: 621, 176, and 233 mmol m⁻², respectively (Table 3). Walligora (1996) reported that rapeseed GSLs reduced seed germination rate and seedling growth of lettuce. Brown and Morra (1996) reported that volatiles from intact roots (5.5 g in dry weight) of rapeseed inhibited the germination of lettuce. The germination rate was about 30% of control on 8.5-cm ×8.5-cm blotter paper (the area was 72.25 cm²). The major GSL in the roots was gluconasturtiin (4.3 μmol g⁻¹ dry wt). Therefore, the glucoraphanin content in the sample was 24.75 μmol and the amount of gluconasturtiin per unit area was roughly 3.4 mmol m⁻². On the other hand, the observed amount of gluconasturtiin in the roots of seedlings was 233 mmol m⁻². These results suggest that the GSLs in volunteer rapeseed seedlings affected the growth of succeeding crops in rhizosphere.

Kirkegaard et al. (2001) reported on the biofumigational functions of GSLs originating from roots. Rumberger and Marschner (2004) reported that the rhizosphere bacterial community composition was correlated with the GSL concentrations in roots. Höglund et al. (1991) reported that GSLs in cortical parenchyma cells coexisted with the enzyme myrosinase or thioglucoside glucohydrolase, and proposed that the enzymes had a nonspecific defense role. Rapeseed seedlings had many small roots and their surface area was large, and this could be one of the reasons for the high GSL content in the roots of rapeseed seedlings. Our results suggest that the GSLs in the roots remaining after harvest and in the leaves and roots of volunteer seedlings are the major cause of the suppression of the growth of the subsequent crop. The GSLs in the dropped seeds of single-low cultivars could also contribute to the suppression. These inhibitory effects might be controlled by decreasing the harvest losses and by removing root residues from the field. The next step is to elucidate how these GSLs suppress the growth of the subsequent crop.

Acknowledgments

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