Changes in the contents of glucosinolates during crop development in different parts of rapeseed varieties

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Abstract

Changes in the amounts of individual glucosinolates (GSL) in Japanese rapeseed (*Brassica napus* L.) varieties were measured in the vegetative and reproductive tissues. Three zero erucic cultivars (Asakanonatane, Nanashikibu, Kizakinonatane) and one double-low cultivar (Kirariboshi) were used. The GSL contents were largely different depending on the plant parts, developing stage and cultivars. Progoitrin and gluconapin were mainly found in the seeds. Their contents were different among cultivars. In the non double-low (single-low) varieties, their contents did not change or increased to some extent with maturing. And the double-low variety, Kirariboshi, contained almost no progoitrin or gluconapin throughout its growth stages. Glucobrassicanapin, glucobrasscin and gluconasturtiin were found in all four varieties, mainly detected in their roots. Even in the double-low variety, Kirariboshi, they were detected. The content of glucobrassicin in the roots tended to decrease with maturing. And that of gluconasturtiin was similar in all cultivars at harvest time. But the patterns of its change were different among the cultivars. In Kizakinonatane, its content was decreased with maturing. In Asakanonatane, Kirariboshi and Nanashikibu, it did not change largely.These results showed that GSL contents in Japanese rape (*Brassica napus* L.) were different among plant parts, maturing stage and cultivars. And it was found that even a double-low variety contained some GSL in the roots. More information is needed for clarifying if the GSL in the roots have some effects or physiological function on the subsequent crops.

Key words: *Brassica napus* L., glucosinolate (GSL), plant parts, cultivar

Introduction

GSL are contained in parenchyma of *Brassica napus* L.. Because its hydrolysis products, thiooxazolidone and isothiocyanate were toxic (Astwood E. B. *et al*.1949, Kawagishi S.1985), low GSL varieties were bred. Regarding its hydrolysis products, the biofumigation effect to soil-borne pathogens was reported (Bellostas N. *et a*l. 2004). And Smith B. J. *et al*. (2002) reported that hydrolysis of glucosinolates in root tissues affected to growth of fungi and oomycetes.In our laboratory we are studying rotational cropping system using rape, barley and sunflower. In the field test, the growth of sunflower after rape was poorer than after barley. One of the aims of the present study was to clear the mechanisms of this phenomenon and therefore, we analyzed the contents of GSLs in stems, leaves, roots and reproductive organs at different growth stages for studying of its effects to succeeding crops.

Material and methods

Plant materials The rapeseed of "Asakanonatane (Norin No.46)", "Kizakinonatane (Norin No.47)" and "Nanashikibu (Norin No.49)" were zero erucic cultivars (single-low cultivars). And "Kirariboshi (Norin No.48)" was zero erucic and low seed-glucosinolate cultivar (double-low cultivar).*Field experiments* Field experiments were conducted in 2005 and 2006 at the experimental field station of the National Agricultural Research Center (Tsukuba, Japan) on a rotational paddy field in upland conditions. The seeds were sown in 30 × 12m plots per one cultivar. They were sown in the rows 0.3m apart. The space between plants was 5cm. Fertilizer mixture of 106-5-94 kg N, P_2O_5, K_2O, ha^{-1} was uniformly broadcast over the experimental area. Weeds were removed manually. *Sampling* Samples were taken in three replications at April 25, May 9, 23, June 6, 20 in 2005, and at April 14, 25, May 23, June 20 in 2006. They were separated to leaf, seed, pod, stem, and roots immediately after the sampling, and frozen in liquid N_2 before freeze-drying.*Glucosinolate analysis* Dry samples were milled to fine powder before GSL analysis. GSL extraction and determination were performed as previously described (Ishida *et al*. 1995) using sinigrin as an extraction standard. Separation and detection of desulphoglucosinolates were performed using a Shimazu SPD-10Avp HPLC (Shimazu, Tokyo, Japan) fitted with a 4.6 × 250mm i.d. Intersil ODS-3 (particle size 5μm) column (Shimazu, Tokyo, Japan) and the eluate was detected at 228nm by a UV detector SPD-10AVvp (Shimazu, Tokyo, Japan). Analyses were done in two or three replications for each sample. The glucosinolates were identified

Results

We defied that the major GSL, progoitrin and gluconapin, were contained mainly in seeds and pods (Table). The content of progoitorin in seeds and pods was kept high and that in other parts decreased with maturing (Fig. 1). The content of gluconapin in seeds and pods was once decreased and then increased with maturing. And in other parts it decreased with maturing. About 4-hydroxy-glucobrassicin, the content in seeds and pods was high at about harvest time. In other parts, it

didn't change and was kept low. Glucobrassicin was mostly present in the roots. Its content in roots tended to decrease with maturing. At harvest time, the contents of glucobrassicin and gluconasturtiin in roots between single- and double-low cultivars were not different significantly at the 5% error level (Table, Fig. 2.). The changing patterns of their contents were almost similar in 2005 and in 2006 (data not shown).

Discussion

The changes in the GSL concentrations during crop development were reported by Fieldsend and Milford (1994). It was reported there were differences in the GSL concentration among cultivars and plants parts (leaves, stems, buds, pods and seeds). In this paper, the contents of GSL in the roots were also analyzed. The major finding of this report was that the glucobrassicin and gluconasturtiin are mainly contained in the roots, and their contents in Kirariboshi, a double-low variety, was as high as in the single-low varieties (except gluconasturtiin in Kizakinonatane). About glucosinolates from roots, Kirkegaard *et al.* (2001) reported their biofumigation function. Rumberger and Marschner (2004) reported that rhizosphere bacterial community composition was correlated with the glucosinolate concentration in roots. Probably for these eco-physiological functions, even the double-low cultivar maintained some levels of glucosinolate contents in plant parts (including roots) other than seeds.

The glucobrassicin and gluconasturtiin in the roots could play the suppressing role on the growth of the succeeding crops even after the double-low rapeseed such as 'Kirariboshi'.

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Table Differences in the contents of major glucosinolates in plat parts at harvest time in 2005.

Plant parts	Cultivars	Progoitrin	Gluconapin	4-Hydroxy-Glucobrassicin	Glucobrassicanapin	Glucobrassicin	Gluconasturtiin
		μ mol/g D.W.					
Seeds&Pods	Kizakinonatane	12.37±0.03**	6.12±0.6**	0.22±0.03	n.d.	0.03±0.01	n.d.
	Kirariboshi	0.17±0.00	0.09±0.0	0.65±0.13*	0.59±0.09**	n.d.	n.d.
Stems	Kizakinonatane	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Kirariboshi	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Roots	Kizakinonatane	0.31±0.12	0.14±0.03*	n.d.	n.d.	0.67±0.08	0.38±0.03
	Kirariboshi	n.d.	n.d.	n.d.	0.34±0.12	1.50±0.66	0.34±0.00

Means±SD and represented as relative values to sinigrin (internal standard). n.d.=not detected. Means of about three replicates±S.E. of the mean.

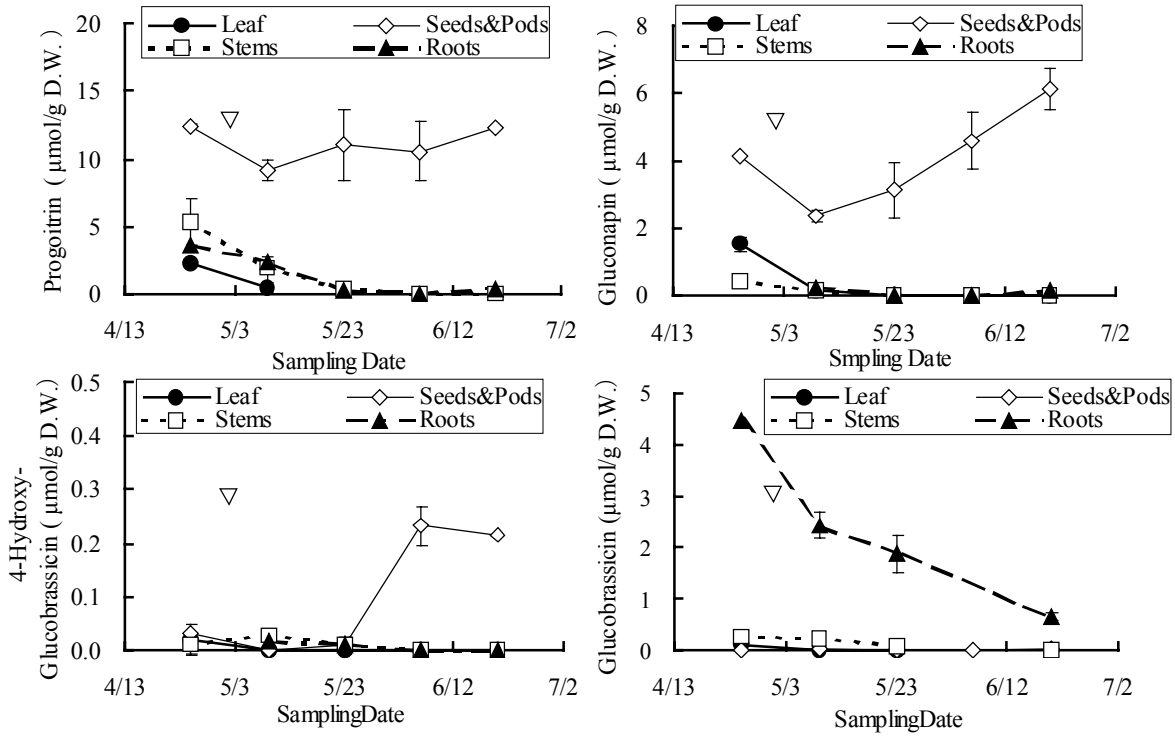

Fig. 1. Changes in the contents of major glucosinolate in different plat parts in 2005 (Kizakinonatane).
Means of about three replicates ± S.E. of the mean. ▽ Termination of flowering

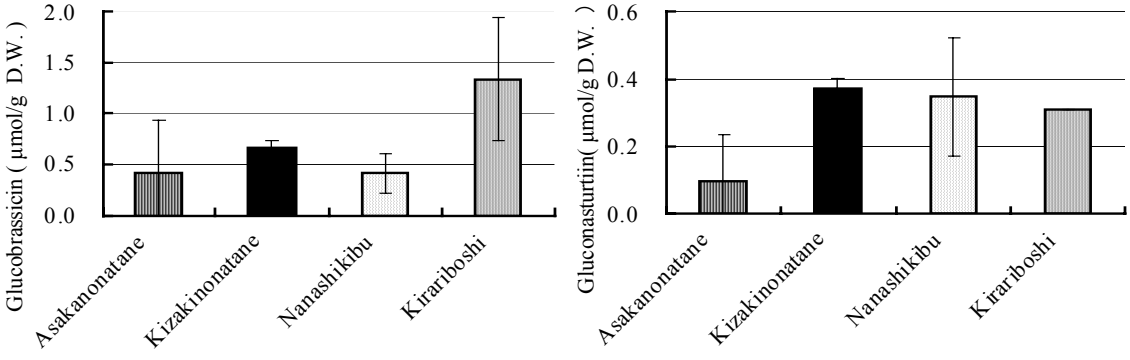

Fig. 2. The contents of glucosinolates (glucobrassicin and gluconasturtiin) in roots at harvest time in 2005.
Means of about three replicates ± S.E.of the mean.