ANALYSIS OF VEGETATIVE TISSUE AS A MEANS OF FACILITATING SELECTION FOR SEED GLUCOSINOLATE COMPOSITION IN BRASSICA

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Glucosinolate analysis of vegetative tissues has been proposed as a screening tool for plant breeding. Johnston and Gosden (1975) suggested using glucosinolate analysis of leaves to select for indole glucosinolates in Brassica oleracea L. Correlation between the glucosinolate content of vegetation and seed led Jurges (1978, 1982) to propose using glucosinolate analysis of leaves to select for both aliphatic and indole glucosinolate content in B. campestris L. and B. napus rapeseed. Xu et al. (1983) also proposed using analysis of rapeseed leaves to select for glucosinolate content of the seed. For a number of years in our laboratory analysis of buds has been used to select for glucosinolate content in rapeseed. Although this procedure benefits from the fact that the glucosinolates tend to be concentrated in the buds, it suffers because waiting till bud formation leaves little time for analysis and selection for crossing purposes. It was of interest, therefore, to further examine the possibility of screening for glucosinolate content on the basis of leaf analysis. Of particular interest was to examine the possibility of screening for allyl glucosinolate in B. juncea (L.) Coss. mustard. Comparative tests have shown that B. juncea mustard can outyield commercially grown Canadian canola cultivars due to better drought tolerance. Brassica juncea mustard also has earlier maturity than current B. napus cultivars and has superior resistance to shattering and disease. Although low erucic acid lines have been selected, it remains to develop a low glucosinolate B. juncea.

In order to determine the degree to which sampling procedures must be standardized, variation in allyl glucosinolate of B. juncea cv. Domo was measured in individual plant parts during development. B. juncea seed was sown in pots containing a soil-free medium (Stringam 1971) and grown in a growth cabinet with an 18 hour photoperiod, light intensity 250 uE/min.^{2}sec., and a 20/15°C light/dark temperature cycle. Four plants were sacrificed every 3 to 4 days and cotyledons, individual leaves, main stem divided into lower, middle and upper portions, inflorescence, pods and seed collected separately when present, frozen, freeze-fried and weighed. The freeze-dried samples were ground, subsampled and analyzed for allyl glucosinolate by gas chromatography of trimethylsilyl derivatives using benzyl glucosinolate as internal standard (Daun and McGregor 1981).

Allyl glucosinolate in the cotyledons was observed to decline from 7 days after seeding (DAS), the earliest date

sampled, when content was expressed on a plant part basis (Fig. 1a).

Allyl glucosinolate in the leaves was observed to increase reaching a maximum at 43 to 47 DAS, the time allyl glucosinolate began to accumulate in the seed. Leaves on the lower part of the plant subsequently senesced without a decline in allyl glucosinolate while leaves higher up showed a decline in glucosinolate content.

Expressed on a gram dry weight basis, the concentration of allyl glucosinolate in leaves two to six increased with development and peaked at 43 to 47 DAS but leaves higher up on the plant had their highest concentration at 27 DAS, the earliest date sampled, and declined thereafter (Fig. 1b). The higher the leaf on the plant the higher the concentration at 27 DAS. The concentration of allyl glucosinolate in the stem was also highest at the earliest date sampled and declined thereafter (Fig. 1c). The concentration in the pods declined as the concentration in the seeds increased. These variations indicate that for maximum prediction accuracy sampling should be restricted to the same leaf taken at the same stage of development.

Decline in allyl glucosinolate content of the leaves after 43 to 47 DAS may be associated with an increase in glucosinolate content in the generative parts of the plant. Rodman and Louda (1984) suggested that a decline in aliphatic glucosinolate content of leaves of Caramine cordifolia when the plant went into flower may be associated with flowering. Cole (1978, 1980) also noted that changes in glucosinolate content seemed to be related to biologically significant events. Whether the changes in allyl glucosinolate in B. juncea represent synthesis and degradation, or translocation, remains to be determined. Increasing allyl concentration at 23 to 31 DAS in leaves progressively higher on the plant is consistent with reports that glucosinolate concentration tends to be highest in young meristematic tissues and declines with age (Kutacek et al 1957; Bergmann 1970). This gradient in allyl concentration may be indicative of translocation of glucosinolate upwards in the plant. Stems do not appear to accumulate relatively large amounts of allyl glucosinolate. Inflorescences and pods, in their early stages of development, and particularly seed, appear to be efficient sinks for allyl glucosinolate accumulation.

Sampling of the second leaf at the four leaf stage of development, was found to allow sufficient time for analysis and selection prior to flowering. It was also found that screening could be further facilitated by taking leaf disks from the blade and hot water extracting the glucosinolate without grinding. Strong correlations were observed for both allyl and 3-butenyl glucosinolate in a breeding line (60073) which contained both glucosinolates in approximately equal amounts (Fig. 2).

Preliminary data indicates that in addition to allyl and 3-butenyl glucosinolate in B. juncea leaves, 4-hydroxybenzyl glucosinolates in the leaves of B. hirta Moench and 3-butenyl glucosinolate in the leaves of B. campestris subspecies sarson leaves can be readily measured (Table 1). However, the content of 3-butenyl and 4-pentenyl glucosinolate, and, in particular, their hydroxylated analogues, 2-hydroxy-3-butenyl and 2-hydroxy-4-pentenyl glucosinolate, are too low to be easily measured in the leaves of B. campestris and B. napus. This would appear to indicate that screening of vegetative tissues of the rapeseed species has limited application.

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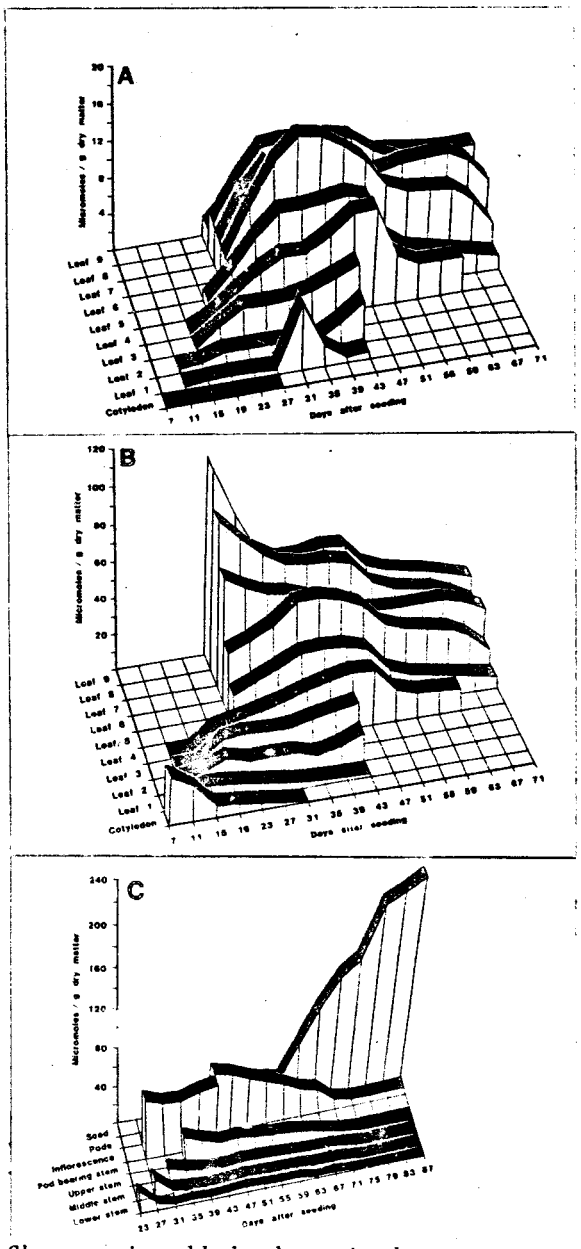

Figure 1. Changes in allyl glucosinolate content of Brassica juncea (L.) Coss. cv. Domo with development. A. Allyl glucosinolate content of cotyledons and individual leaves expressed on a plant part basis. B. Allyl glucosinolate content of cotyledons and individual leaves expressed on a gram dry weight basis. C. Allyl glucosinolate content of the main stem divided into lower, middle and upper portions, inflorescence, pods and seed expressed on a gram dry weight basis.

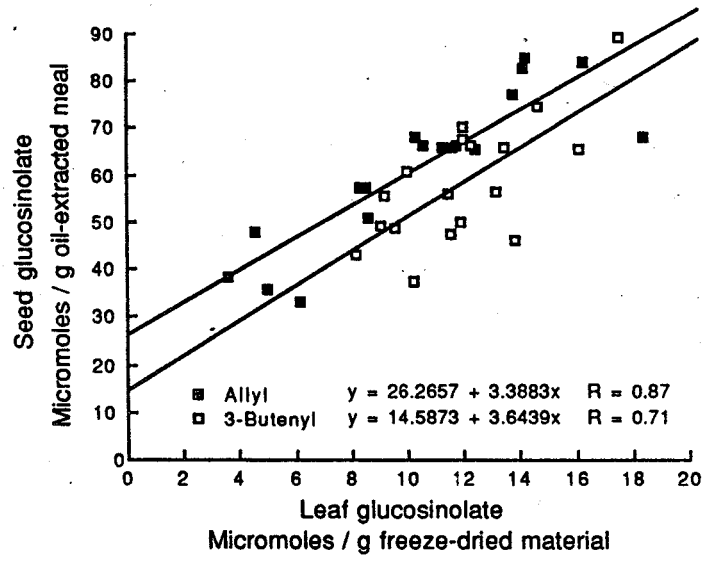

Figure 2. Allyl and 3-butenyl glucosinolate content of the second leaf, analyzed at the four leaf stage of development, and self-pollinated seed of individual plants of Brassica juncea (L.) Coss. line 60037.

Table 1. Glucosinolate content of second leaf, analyzed at the four leaf stage of development and seed of Canadian cultivars and lines of rapeseed (Brassica campestris L. and B. napus L.) and mustard (B. juncea (L.) Coss and B. hirta Moench).

Plant part, species and cultivar	Glucosinolate						
	Allyl	Butenyl	Pentenyl	HO-Butenyl	HO-Pentenyl	HO-Benzyl	Total
Leaf [2]	micromoles / gram freeze-dried material						
B. juncea							
Domo	25.2	0.4	0.1				25.7
ZEM [1]	28.6	0.6					29.2
B. hirta							
Ochre			0.3	0.1		17.1	17.5
B. campestris							
Torch		0.3		0.1			0.4
R-500		15.9		0.1			16.2
Tobin		0.2					0.2
B. napus							
Midas		0.1	0.5	0.8	0.3		1.6
Westar		0.2					0.2
Seed [3]	micromoles / gram oil-extracted meal						
B. juncea							
Domo	185.3	1.3	0.6				187.2
ZEM	168.4						168.4
B. hirta							
Ochre							
B. campestris							
Torch	0.9	36.0	35.2	26.0	5.6		103.7
R-500	0.9	171.9	2.7	2.4	1.9		26.4
Tobin	0.4	7.0	6.1	11.0	1.9		26.4
B. napus							
Midas	1.4	28.4	7.3	98.5	6.6		142.2
Westar	0.7	4.5	0.5	9.7	0.2		15.6

(1) Zero erucic mustard.
(2) Mean of analysis from leaves of three plants.
(3) Mean of duplicate analysis of bulk seeed samples.