Plant Physiol. (1989) 89, 260–263
0032-0889/89/89/0260/04/$01.00/0

Received for publication April 29, 1988
and in revised form August 11, 1988

# Glucosinolate Uptake by Developing Rapeseed Embryos[1]

**Mark Gijzen, Ian McGregor*, and Ginette Séguin-Swartz**

*Agriculture Canada Research Station, Saskatoon, Saskatchewan, Canada S7N 0X2*



## ABSTRACT

**Embryos excised from the seed of rapeseed (*Brassica napus* L.) accumulated glucosinolate from the culture medium. Uptake was saturable, subject to inhibition, varied with the developmental stage of the embryo but correlated with the time of accumulation of glucosinolates *in situ*. It is suggested that a carrier-mediated transport system is operating in the developing embryo.**

During embryogenesis, glucosinolates accumulate in the seed of rapeseed (*Brassica napus* L). At maturity, the embryo comprises from 80 to 90% of the seed dry weight and is the site where most of the glucosinolates contained in the seed are located (7, 14). The subcellular location of the glucosinolates within the embryo is not known for certain, although they are thought to be localized in globoid bodies associated with aleurone grains (17), structures which form within and eventually fill the vacuoles. Inheritance studies (7, 14) and grafting experiments (9) have indicated that seed glucosinolate content is maternally controlled. Whether embryos have any synthetic capability, or simply act as a sink for glucosinolates synthesized in the pod or other plant parts, is not known.

Immature rapeseed embryos germinate when removed from the seed if placed in nutrient medium or water (3). Precocious germination may be prevented by increasing the osmotic potential of the medium or by adding ABA (4, 5). Immature embryos cultured on high osmoticum develop normally in that they continue to synthesize and accumulate embryo-specific storage protein (4). This investigation presents evidence that immature rapeseed embryos cultured on high osmoticum do not synthesize and/or accumulate glucosinolates if glucosinolates are absent from the culture medium, but can take up exogenously supplied glucosinolate in a carrier-mediated fashion. This ability to take up glucosinolate varies with development of the embryo, and may account for glucosinolate accumulation *in situ*.

## MATERIALS AND METHODS

### Plant Materials

Rapeseed (*Brassica napus* L. cv Golden) plants were grown in soil-free medium (15) in growth cabinets under an 18 h photoperiod with a photosynthetic photon flux density of 250 $\mu$E $m^{-2}$ $s^{-1}$ and a 22°C/18°C light/dark cycle. Illumination was provided by a combination of fluorescent and incandescent lights. Flowers were hand pollinated and the date of pollination was recorded.

[1] Contribution No. 981.

### Accumulation Experiments

Accumulation of glucosinolate *in situ* was examined from 16 to 40 DAP[2] by excising embryos from seeds at 2 to 4 d intervals. Accumulation of glucosinolates *in vitro* was examined by aseptically excising embryos from the seeds and culturing 10 per Petri plate (6 × 1.5 cm) in 5 mL of solid Monnier's culture medium (12) (filter sterilized, 0.4% agarose) according to the method of Crouch and Sussex (3) with and without the addition of 1.0 mM 2-propenyl-, 3-butenyl-, or benzylglucosinolate. Potassium salt of 2-propenylglucosinolate (sinigrin) was purchased from Sigma. The tetramethylammonium salts of 3-butenylglucosinolate and benzylglucosinolate were purified from the seeds of rapeseed (*Brassica campestris* L. cv R500) and nasturtium (*Tropaeolum majus* L.), respectively, by the method described by Hanley *et al.* (6). Each plate constituted a sample.

Glucosinolate uptake by embryos over a 26 h period was examined by excising embryos from seeds, 10 to 20 per sample, and placing in 5 mL of medium containing 0.35 M sucrose, 10 mM Mes/KOH buffer (pH 6.5), and 4.0 mM benzylglucosinolate. Benzylglucosinolate concentrations in the range of 0.1 to 15 mM were used to study the effect of glucosinolate concentration on uptake. The protonophores CCCP and 2,4-DNP, the respiratory chain inhibitor $NaN_3$, and the ATPase and PPase cofactor $Mg^{2+}$ (in the form of $MgSO_4$) were added to the incubation medium to determine if these compounds have inhibitory or stimulatory effects on glucosinolate uptake.

To determine the relationship between glucosinolate uptake *in vitro* and glucosinolate accumulation by embryos during development *in situ*, uptake by embryos excised at various stages of development was compared to total glucosinolate content of embryos growing *in situ*. Uptake was assayed in embryos incubated in a medium containing 0.35 M sucrose, 10 mM Mes/KOH buffer (pH 6.5), and 1.0 mM 2-propenylglucosinolate as substrate.

Uptake experiments were performed under fluorescent lights and at room temperature, 24°C and, with the exception of the variable time experiments, for 24 h. Uptake was expressed on a FW basis as $\mu$mol (g FW)$^{-1}$ $h^{-1}$. All inhibitors were obtained from Sigma.

### Glucosinolate Determinations

Embryos were washed with 10 mL of distilled water, blotted dry on filter paper, weighed, and placed in test tubes containing 3.0 mL of hot distilled water heated in a boiling water

[2] Abbreviations: DAP, days after pollination; FW, fresh weight; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; 2,4-DNP, 2,4-dinitrophenol.

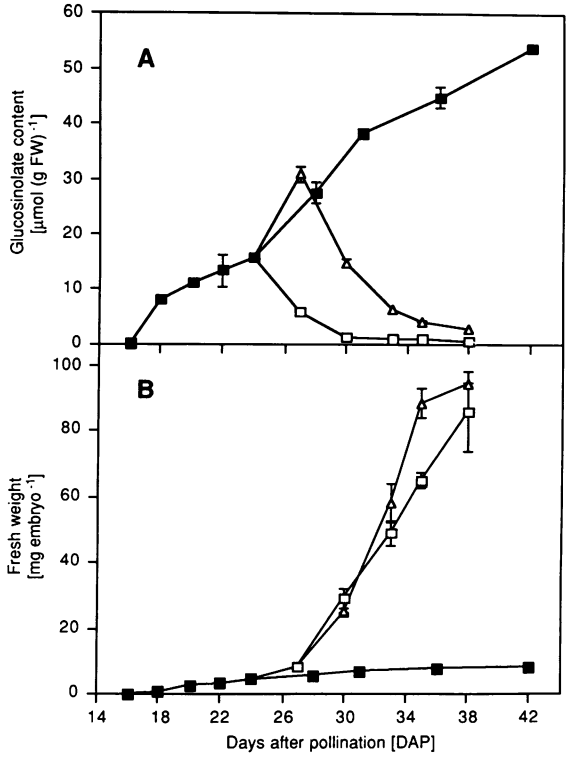


**Figure 1.** Total glucosinolate content (A) and fresh weight (B) of Golden rapeseed embryos. Embryos developing *in situ* (■), embryos removed at 24 DAP and cultured *in vitro* on Monnier's medium (□), embryos removed at 24 DAP and cultured *in vitro* on Monnier's medium + 1.0 mM 2-propenyl glucosinolate (△). Values are means of duplicate determinations; bars represent the range.

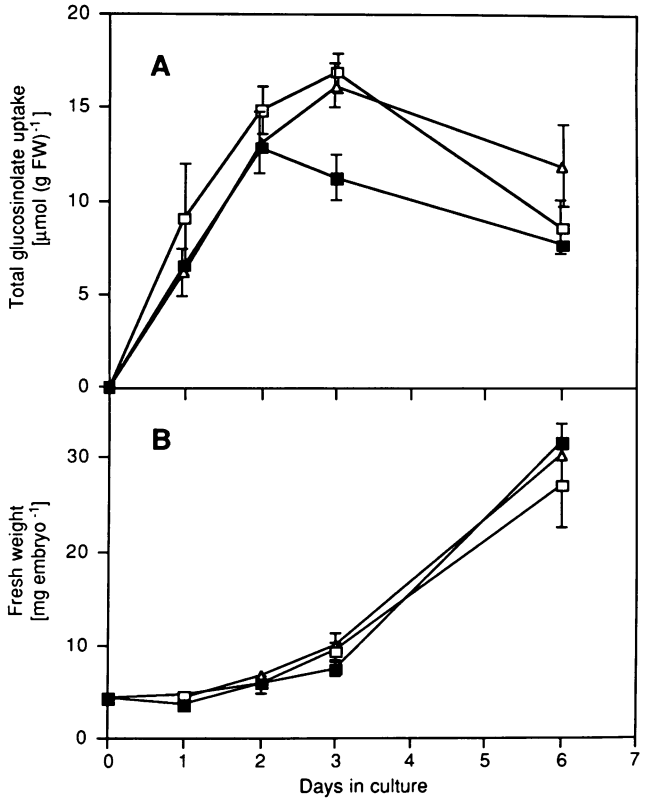


**Figure 2.** Glucosinolate uptake (A) and fresh weight (B) of Golden rapeseed embryos removed at 24 DAP and cultured *in vitro* on Monnier's medium. Embryos cultured *in vitro* with media additions of: 1.0 mM 2-propenyl-glucosinolate (□), 1.0 mM Benzyl-glucosinolate (■), 1.0 mM 3-butenyl-glucosinolate (△). Values are means of duplicate determinations; bars represent the range.

bath. Samples were boiled for 5 min and then allowed to cool before homogenization with a Polytron high frequency mechanical and ultrasonic homogenizer. The shaft of the Polytron was rinsed with a 3.0 mL aliquot of distilled water, which was pooled with the sample. Protein and sulfate were precipitated with the addition of 0.5 mL of 0.06 M barium acetate/lead acetate solution. Samples were mixed and centrifuged (2000 *g*) for 10 min. Glucosinolates were purified by isolation on a DEAE-Sephadex column and eluted with distilled water after desulfation with sulfatase (Sigma) (16). Desulfoglucosinolates were separated on a C-18 reverse phase HPLC column using a water-acetonitrile gradient (11), and UV detection at 226 nm. Quantitation was achieved by using response factors determined by reacting thymol with individual desulfoglucosinolates isolated by HPLC (2, 10). Accumulation of 2-propenyl- and 3-butenylglucosinolate were measured as the difference in glucosinolate content μmol (g FW)$^{-1}$ of the embryo at sampling time and the time culturing was initiated because Golden rapeseed embryos may contain trace amounts of 2-propenylglucosinolate and substantial amounts of 3-butenylglucosinolate. Accumulation of benzylglucosinolate was measured as the content at the time of sampling, since Golden rapeseed embryos do not contain this glucosinolate.

## RESULTS AND DISCUSSION

Accumulation of glucosinolates *in situ* commenced at 16 DAP and rose rapidly for the next 24 DAP (Fig. 1). Embryos removed from the pod at 24 DAP and cultured *in vitro* failed to show a similar accumulation of glucosinolate. Addition of 2-propenylglucosinolate to the medium resulted in rapid uptake of this compound. After 3 d of culture, half of the 2-propenylglucosinolate originally in the medium could be accounted for in the embryos. The concentration of glucosinolate in these embryos was in excess of 20-fold of that in the medium after 3 d of culture. Depletion of glucosinolate supply in the medium and large increases in FW of the embryos cultured longer than 3 d resulted in an effective decline in glucosinolate concentration in the embryos after 3 d of culture. Embryos also accumulated 3-butenyl- or benzylglucosinolate when cultured in the presence of these glucosinolates (Fig. 2). Of the glucosinolates tested, only 3-butenylglucosinolate is present in substantial amounts *in situ*; 2-propenylglucosinolate is present in trace amounts, and benzylglucosinolate is absent. That all these glucosinolates were accumulated by the embryos with similar efficiency indicates that the factor(s) responsible for their accumulation may have little specificity toward the side chain. Depletion of glucosinolate supply in the medium and large increases in FW of the embryos cultured longer than 3 d resulted in an effective decline in glucosinolate concentration in the embryos after 3 d of culture. This was substantiated by subculturing the embryos after 3 d in fresh medium with and without 2-propenylglucosinolate (Fig. 3). Without added glucosinolate

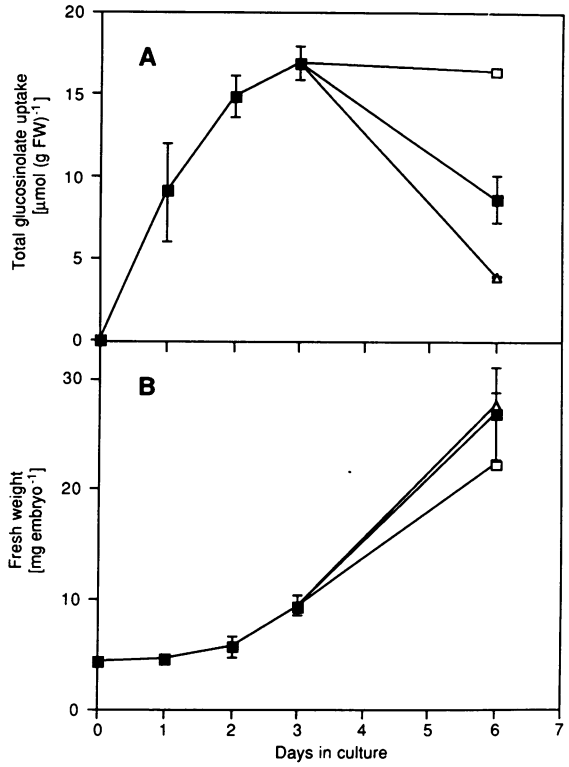


**Figure 3.** Glucosinolate uptake (A) and fresh weight (B) of Golden rapeseed embryos removed at 24 DAP and cultured *in vitro* on Monnier's medium. Embryos cultured *in vitro* in the presence of 1.0 mM 2-propenyl-glucosinolate (■), embryos transferred to fresh glucosinolate containing medium after 3 d *in vitro* (□), embryos transferred to fresh medium without glucosinolate after 3 d *in vitro* (△). Values are means of duplicate determinations; bars represent the range.

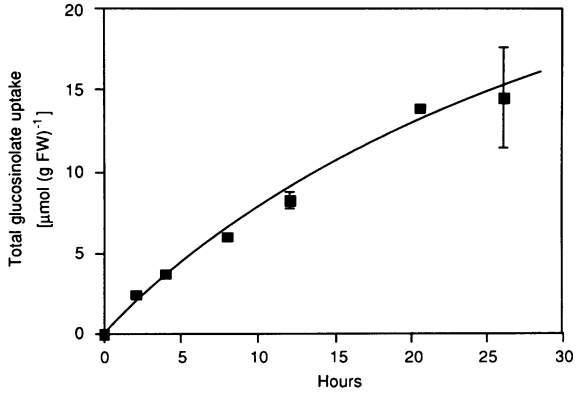


**Figure 4.** Time course for benzylglucosinolate uptake by Golden rapeseed embryos at 21 DAP. Initial glucosinolate concentration in the media was 4.0 mM. Values are means of duplicate determinations; bars represent the range.

in the medium, the glucosinolate content of the embryos declined, while in the presence of added glucosinolate, although no further increase was observed, the embryos maintained a 15- to 20-fold higher concentration of glucosinolate relative to the medium.

Uptake of benzylglucosinolate was not linear over the 26 h period investigated, although the deviation from linearity was slight (Fig. 4). The uptake showed a saturation effect with

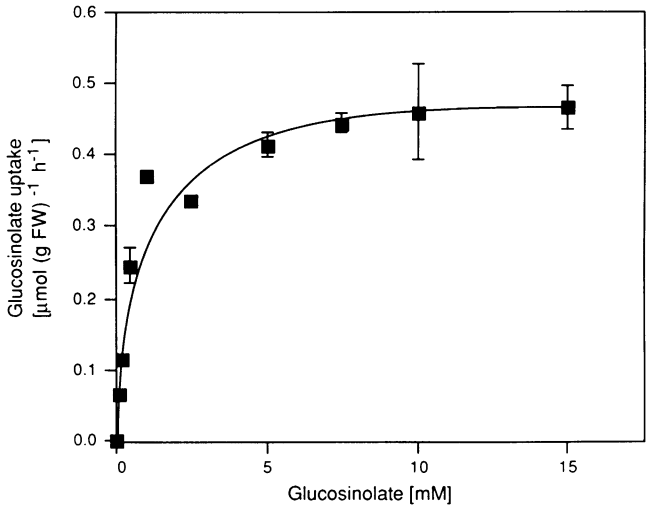


**Figure 5.** Influence of benzylglucosinolate concentration on the uptake of benzylglucosinolate by Golden rapeseed embryos at 21 DAP. Values are means of duplicate determinations; bars represent the range.

**Table I.** *Effect of CCCP, 2,4-DNP, $NaN_3$, and $MgSO_4$ on Benzylglucosinolate Uptake in Embryos 21 DAP*

Mean and range (in parentheses) of duplicate determinations. The experiments have been repeated with similar results.

| Treatment | Concentration | Relative Uptake of Glucosinolate |
|---|---|---|
| | *mM* | % |
| Control | | 100[a] |
| CCCP | 0.01 | 24.5 (2.4) |
| | 0.1 | 22.3 (0.6) |
| 2,4-DNP | 0.1 | 19.6 (3.2) |
| | 1.0 | 7.9 (0.6) |
| Control | | 100[b] |
| $NaN_3$ | 0.5 | 20.4 (2.2) |
| | 2.0 | 19.4 (2.8) |
| $MgSO_4$ | 2.0 | 191.8 (19.6) |

[a] The incubation medium contained 4.0 mM benzylglucosinolate, 0.35 M sucrose, 10.0 mM Mes/KOH buffer (pH 6.5), and 1% DMSO. The control rate was 0.57 (0.056) µmol g FW$^{-1}$ h$^{-1}$. [b] The incubation medium contained 4.0 mM benzylglucosinolate, 0.35 M sucrose, and 10.0 mM Mes/KOH buffer (pH 6.5). The control rate was 0.36 (0.038) µmol g FW$^{-1}$ h$^{-1}$.

increasing substrate concentrations (Fig. 5). Depletion of substrate from the medium did not contribute to the saturation effect since no more than 5% of total glucosinolate originally in the medium could be accounted for in the embryos at any given concentration. Uptake of benzylglucosinolate was inhibited by CCCP, 2,4-DNP, and $NaN_3$ and was stimulated by $Mg^{2+}$ (Table I). Whether the inhibitors were simply effective in killing the embryos or had more specific effects on transport of glucosinolate is not known.

Comparison of glucosinolate uptake by embryos excised at various stages of development with the glucosinolate content of embryos growing *in situ* showed that uptake *in vitro* rose rapidly from 16 to 20 DAP, peaked from 20 to 22 DAP, and declined slowly thereafter, exhibiting only residual activity at 36 DAP and beyond, while glucosinolate accumulation *in*

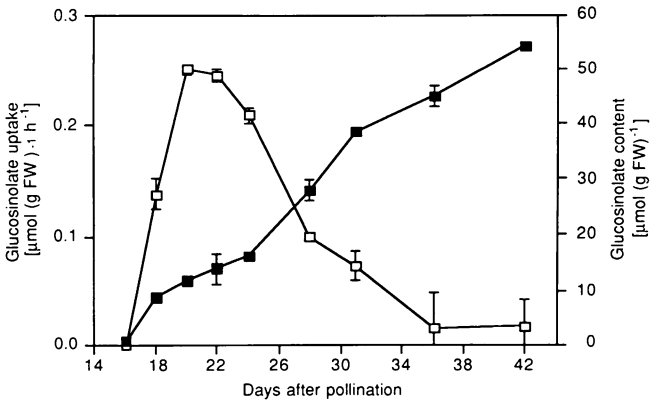


**Figure 6.** Glucosinolate content *in situ* (■) and uptake *in vitro* using 1.0 mM 2-propenyl glucosinolate as a substrate (□) of Golden rapeseed embryos from 16 to 42 DAP. Values are means of duplicate determinations; bars represent the range.

*situ* commenced at 16 DAP, rose rapidly, then leveled off after 30 DAP (Fig. 6). Uptake *in vitro*, therefore, appears concomitant with glucosinolate accumulation *in situ*. The final glucosinolate content of embryos of *B. napus* cv Golden, 40 to 60 μmol (g FW)$^{-1}$, may depend on the position of the silique on the plant as has been observed for *B. campestris* (13).

The presence of glucosinolates in the seed of rapeseed affects the feeding value of the seed and of the meal after oil extraction. In addition to reducing palatability, in nonruminant animals hydrolysis products can cause metabolic disorders, poor feed efficiencies, and reduced weight gain (1). To date, attempts to lower the glucosinolate content of rapeseed and other *Brassicas* by conventional sexual selection have resulted in an altered ability by the plant to synthesize glucosinolates (8). The results of the present study, along with the knowledge that embryo glucosinolate content is determined maternally (7, 9, 14), lead us to speculate that glucosinolates are accumulated in embryos *in situ* through an active transport process. Isolation by sexual breeding, *in vitro* cellular cultivation, or molecular genetics of rapeseed lines which differ in the ability of the maturing embryo to accumulate glucosinolate may be an alternate means of reducing seed glucosinolate content in this important oilseed crop.

## LITERATURE CITED

1. **Bell JM** (1984) Nutrients and toxicants in rapeseed meal: a review. J Anim Sci **58:** 996–1010
2. **Brzezinski W, Mendelewski P** (1984) Determination of total glucosinolate content in rapeseed meal with thymol reagent. Z Pflanzenzuecht **93:** 177–183
3. **Crouch ML, Sussex IM** (1981) Development and storage-protein synthesis in *Brassica napus* L. embryos *in vivo* and *in vitro*. Planta **153:** 64–74
4. **Finkelstein RR, Crouch ML** (1986) Rapeseed embryo development in culture on high osmoticum is similar to that in seeds. Plant Physiol **81:** 907–912
5. **Finkelstein RR, Tenbarge KM, Shumway JE, Crouch ML** (1985) Role of ABA in maturation of rapeseed embryos. Plant Physiol **78:** 630–636
6. **Hanley AB, Heaney RK, Fenwick GR** (1983) Improved isolation of glucobrassicin and other glucosinolates. J Sci Food Agric **34:** 869–873
7. **Josefsson E** (1970) Glucosinolate content in seed of *Brassica napus*, *B. campestris*, and *Sinapis alba* as affected by seed part, inheritance, and environment. *In* Pattern, content, and biosynthesis of glucosinolates in some cultivated Cruciferae. Thesis, University of Lund, Lund, Sweden, pp 19–22
8. **Josefsson E** (1973) Studies of the biochemical background to difference in glucosinolate content in *Brassica napus* L. III. Further studies to localize metabolic blocks. Physiol Plant **29:** 28–32
9. **Lein KA** (1972) Genetische und physiologische untersuchungen zur bildung von glucosinolaten in rapssamen: Lokalisierung des haupt-biosyntheseortes durch Pfropfungen. Z Pflanzenphysiol **67:** 333–342
10. **McGregor DI** (1985) Determination of glucosinolate in *Brassica* seed. Eucarpia Cruciferae Newsletter 10, pp 132–136
11. **Michinton I, Sang J, Burke D, Truscott RJW** (1982) Separation of desulfoglucosinolates by reverse-phase high-performance liquid chromatography. J Chromatogr **247:** 141–148
12. **Monnier M** (1976) Culture in vitro de l'embryo immature de *Capsella bursa pastoris* Moench. Rev Cytol Biol Veg **39:** 1–120
13. **Rahman MH, Stølen O, Sørensen H** (1986) Glucosinolate content in seeds from silique at different position of *Brassica campestris*. Acta Agric Scand **36:** 318–324
14. **Röbbelen G, Thies W** (1980) Variation in rapeseed glucosinolates and breeding for improved meal quality. *In* S. Tsunoda, K Hinata, C Gómez-Campo, eds, Brassica Crops and Wild Allies. Japan Scientific Society Press, Tokyo, pp 285–297
15. **Stringam GR** (1971) Genetics of four hypocotyl mutants in *Brassica campestris* L. Heredity **62:** 248–250
16. **Thies W** (1979) Detection and utilization of a glucosinolate sulfohydrolase in the edible snail, *Helix pomatia*. Naturwissenschaften **66:** 364–365
17. **Yiu SH, Collins FW, Fulcher RG, Altosaar I** (1984) Chromatographic and microscopic detection of glucosinolates in rapeseed using *N*-2,6-trichloro-*p*-benzoquinoneimine. Can J Plant Sci **64:** 869–878