GLUCOSINOLATES IN A CELL SUSPENSION CULTURE OF BRASSICA JUNCEA (L.) COSS.

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Glucosinolates are natural plant products which occur in eleven plant families including Brassicaceae (McGregor et al. 1983). Glucosinolates have been detected in vegetative tissue of seedlings and mature plants, including root, hypocotyl, cotyledons, shoot apex, leaf and pod tissue, as well as in developing and mature seed. Few reports have been published on the glucosinolate profiles of callus and suspension cell lines derived from glucosinolate containing tissue. Aliphatic and aromatic glucosinolates, or their hydrolysis products, have been detected in suspension cell cultures of garden nasturtium, Tropaeolum majus L., and the dyer's rocket, Reseda Luteola L. (Kirkland et al. 1971), as well as in callus cultures of dyer's woad, Isatis tinctoria L. (Goetz and Schraudolf 1983), and flixweed, Descurainia Sophia (L.) Webb (Afsharypuor and Lockwood 1985). Glucosinolates were not demonstrated in cell suspension cultures of horseradish, Armoracia lapathifolia Gilib., white mustard, Sinipis alba L., edging candytuff, Iberis sempervirens L., watercress, Nasturtium officinale R. Br., and rocket, Eruca sativa Lam. (Kirkland et al. 1971). Myrosinase activity, however, was observed in cell cultures of these species.

Within the Brassica species, glucosinolates have been detected in callus cultures belonging to the oleracea group, broccoli, kohlrabi and cauliflower (Goetz and Schraudolf) 1983), but not in callus cultures of rapeseed, Brassica napus L. (Afzalpurkar 1974; Rogozinska and Drozdowska 1981). In this paper, we report the glucosinolate content and composition of a cell suspension culture of brown mustard, Brassica juncea (L.) Coss.

Brassica juncea cell line 603JC was initiated in August 1985 from mesophyll protoplasts of the cytoplasmic male sterile Varuna line (Dr. I. J. Anand, Agriculture Research Institute, New Delhi, India) which had been cultured according to the method of Kao and Séguin-Swartz (1987). The cell suspension has been subcultured weekly in autoclaved M93 medium (half-strength Murashige and Skoog 1962) macroelements, microelements and vitamins, 2% sucrose, 2 mg/L alpha-naphthaleneacetic acid and 0.1 mg/L benzylaminopurine, pH 5.8, prior to autoclaving). The culture flasks are incubated on a rotary shaker (100 rpm), at room temperature, under 16 hour daily illumination (90 $\mu E/m^2 s$). For this study, 125 mL Erlenmeyer flasks containing 30 mL of culture medium were inoculated with 3 g of cells from a 12 day old culture (stationary phase). At

day 7, the medium from each flask was replaced with an equal volume of fresh medium.

Cell growth was assessed by the packed cell volume method on duplicate samples obtained from different flasks. Desulfo glucosinolates were separated by high performance liquid chromatography according to the method of Minchinton et al. (1982) and quantitated using response factors of McGregor (1985). Glucosinolate content and composition were measured on duplicate samples from different culture flasks.

Following subculturing in fresh medium (day 0), the cell cultures remained in lag phase without any increase in packed cell volume for 7 days (Fig. 1). After replacing the medium at day 7, the cell cultures entered an exponential growth phase increasing substantially in packed cell volume until day 14 when they appeared to enter a stationary phase of growth.

Only indole glucosinolates, 3-indolylmethyl, 4-hydroxy-3-indolylmethyl, 4-methoxy-3-indolylmethyl and 1-methoxy-3-indolylmethyl, could be detected in the cell cultures. The total amount of these glucosinolates was found to vary independently of cell growth commencing to increase at day 2, during the lag phase of growth, and reaching a maximum, when expressed per mL of packed cell volume, at day 8 (Fig. 1). Total indole glucosinolate per flask reached a maximum at day 14 (not shown).

All of the indole glucosinolates, when expressed per mL of packed cell volume, increased prior to the change of medium at day 7, but 4-hydroxy-3-indolylmethyl and 4-methoxy-3-indolylmethyl glucosinolate appeared to peak at day 6 and decline subsequently (Fig. 2). Following transfer to fresh medium at day 7, 4-methoxy-3-indolylmethyl glucosinolate increased substantially while 3-indolylmethyl glucosinolate declined. This decline was then followed by an increase to a maximum at day 10, then a second decline. The content of 4-methoxy-3-indolylmethyl and 1-methoxy-3-indolylmethyl glucosinolate reached a maximum at day 8 then declined while the content of 4-hydroxy-3-indolylmethyl reached a maximum at day 13 then declined.

Cell cultures have rarely been observed to accumulate secondary metabolites, and in those situations when it has been observed, accumulation usually has occurred only after the cells have ceased growing (Ellis 1984; Collinge 1986). It is noteworthy that in the present study accumulation commenced before the exponential growth phase. Also noteworthy is the exclusive occurrence of the indole glucosinolates. Among the Brassicas, the predominant glucosinolates in the seeds are one or more aliphatic glucosinolates, while in the root tissue 2-phenylethyl glucosinolate predominates, and in leaves either aliphatic or indole glucosinolates, or both, may predominate (Sang et

al. 1984). In B. juncea allyl glucosinolate is predominant in vegetative tissues but is accompanied by lesser amounts of the indole glucosinolates (Sang et al. 1984).

The complete absence of allyl glucosinolate from the cell culture appears to indicate that its presence is not essential to the metabolism of the growing plant cell and that the role aliphatic glucosinolates play in the plant, if any, may be phytoprotective. The presence of indole glucosinolates in the cell culture may indicate that they serve an alternate role in plant cell metabolism. The possibility that these compounds are acting as plant hormones, or precursors to plant hormones, was suggested with their first discovery (Kutacek 1964). The hydrolysis of 3-indolylmethyl glucosinolate to indoleacetonitrile and indoleacetic acid has been demonstrated to occur under some conditions (Searle et al. 1982), and is thought to be responsible for the proliferation of growth around the site of clubroot (Plasmodiophora brassicae) infection (Butcher et al. 1974).

Although much is known about the biosynthetic pathway of the glucosinolates (Underhill 1980), details of the pathway to indole glucosinolate are yet to be elucidated. Studies by Mahadevan and Stowe (1970, 1972) with dyer's woad demonstrated that 3-indolylacetaldehyde was an effective precursor to 1-sulfo-3-indolylmethyl and 1-methoxy-3-indolylmethyl glucosinolate, and they suggested that these two glucosinolates were derivatives of 3-indolylmethyl glucosinolate. Alternately, Goetz and Schraudolf (1983) have suggested that the biosynthetic sequence tryptophan to hydroxytryptophan, 4-hydroxy-3-indolylmethyl glucosinolate then 4-methoxy-3-indolylmethyl glucosinolate. Myrosinase hydrolysis is the only pathway by which glucosinolate degradation has been demonstrated (Larsen 1981). However, although the existence of different isoenzymes has been established and it has been shown that different glucosinolates can be degraded at different rates, there appears to be no specificity for different glucosinolates (Larsen 1981). In the present study, changes in the indole glucosinolate profile of the cell culture with development appeared to be somewhat complex. Individual glucosinolates did not vary in concert. The extent to which these changes represent de novo synthesis, degradation or interconversion remains to be determined.

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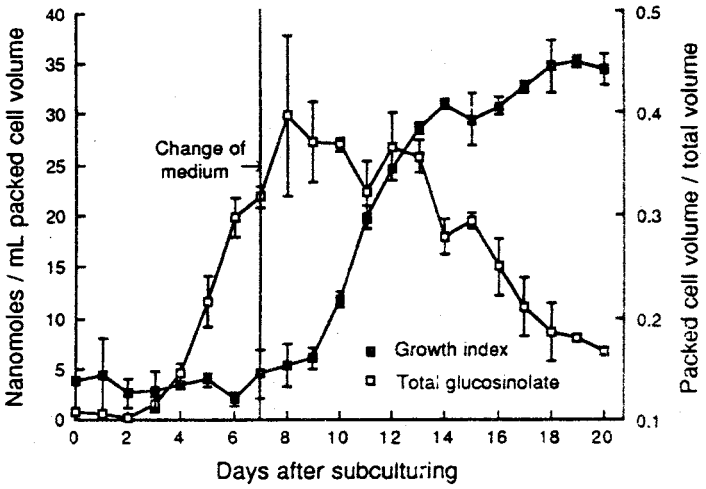

Figure 1. Indole glucosinolate content and growth index of a Brassica juncea (L.) Coss. cell suspension culture subcultured for 21 days.

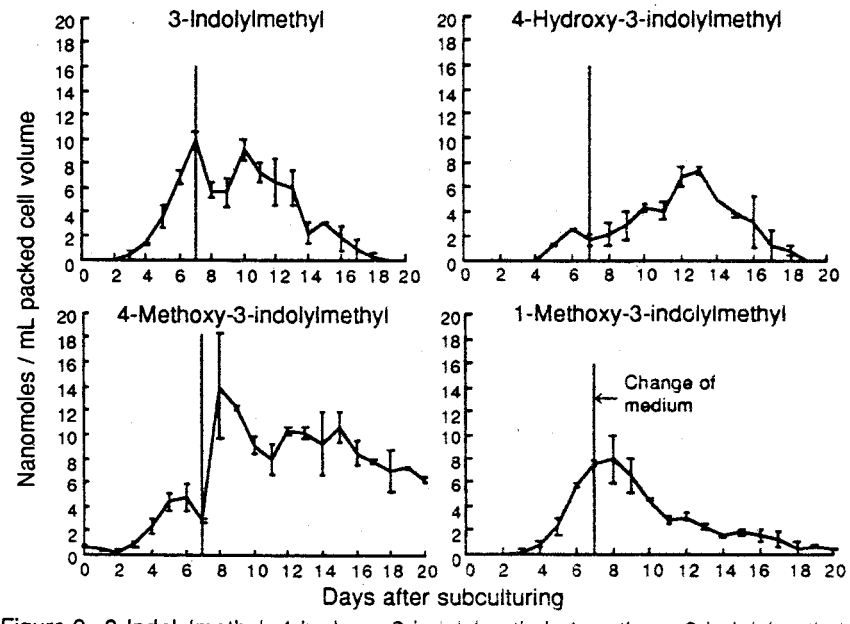

Figure 2. 3-Indolylmethyl, 4-hydroxy-3-indolylmethyl, 4-methoxy-3-indolylmethyl 1-methoxy-3-indolylmethyl glucosinolate content of a Brassica juncea (L.) Coss. cell suspension culture subcultured for 21 days.