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# ISOTHIOCYANATE GLYCOSIDE PRODUCTION BY PLANT TISSUE CULTURES FROM TWO SPECIES OF THE CRUCIFERAE

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Increasing pressure to avoid use of synthetic additives in the food industry has led to an increase in the search for natural flavouring materials. The isothiocyanate glycosides are flavour components which yield the characteristic pungent taste typified by the mustards, as when crushed their volatile aglycones are liberated due to hydrolysis caused by endogenous myrosinase enzyme. Analysis is commonly carried out by hydrolysis of the glycoside, followed by quantitation of the one or more aglycones which are liberated. An extremely sensitive technique has been devised for the *in situ* hydrolysis of the glycoside, followed by solvent extraction and analysis of the aglycones using GC and GC-MS (Lockwood and Afsharypuor 1986). This method allows analysis of mg. quantities of plant material and also simultaneous analysis of fatty acids and hydrocarbons.

To date there has been only limited success in the *in vivo* production of isothiocyanate glycosides by tissue techniques due to very low levels and insensitive techniques. We have established callus and suspension cultures of *Descurainia sophia* (L) Webb ex Prantl and *Alyssum minimum* Willd (Cruciferae).

Cultures were grown on Murashige and Skoog's medium supplemented with 0.2-1.0mg/l auxins plus 0.1 or 0.5mg/l kinetin. Plant and callus of *D. sophia* were found to yield the same major glycosides, namely allyl- and 3-butenyl-glucosinolate, whereas the major glycoside of *A. minimum* plant occurred in callus in only trace amounts (Table 1). Both types of cultures grown in media supplemented with 2,4-dichlorophenoxyacetic acid produced higher glycoside levels than similar cultures supplemented with α-naphthylacetic acid as the auxin source.

These are the first cultures for which glycosidically bound flavour components have been quantified, and their levels are comparable with other volatiles of cell cultures.

Table 1. Levels of isothiocyanate glycosides in whole plants and callus cultures (μg/g dry wt.)*

| | *Descurainia sophia* | | *Alyssum minimum* | |
|---|---|---|---|---|
| | Plant | Callus | Plant | Callus |
| Allyl glucosinolate (Sinigrin) | 243.4 | 1.6 | - | 0.8 |
| 3-Butenyl glucosinolate (Gluconapin) | 148.8 | 0.3 | 1251.0 | Trace |
| 3-Phenylethylglucosinolate (Gluconasturtiin) | - | 0.1 | - | - |
| 7-Methyl thioheptyl glucosinolate | - | - | - | 0.6 |

*levels calculated via analysis of isothiocyanates, nitriles and epithiobutanes.

When cultures of *D. sophia* were grown under temperature stress (4°C) there was an increase in fatty acid levels but no alteration in isothiocyanate glycosides. Sulphate supplementation of media failed to bring about increased levels of isothiocyanate glycoside, however feeding of isothiocyanate aglycone to germinating seedlings did not result in accumulation of aglycone or glycoside.

We have also shown that both callus and suspensions of *D. sophia* continue to accumulate n-alkanes and fatty acids (Afsharypuor and Lockwood, 1985), although it appears that either decreased morphological differentiation or increase in catabolism causes low levels of isothiocyanate glycoside, notably in suspension cultures. These results indicate the ability of plant tissue cultures to accumulate glycosides which yield volatile aglycones, however these results suggest the possibility of endogenous glycoside hydrolysis.

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