Daily Variation in Glucosinolate Concentrations in the Leaves and Roots of Cabbage Seedlings in Two Constant Temperature Regimes

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Abstract: Limited information is available on the glucosinolate variation within the Brassica plant and the relationship between the pattern and concentration of glucosinolates in the aerial parts and the roots has received little attention. Early studies carried out under field conditions have shown that glucosinolate levels may vary considerably throughout a 24 h period. The purpose of the present study was to show whether, under controlled conditions, temperature was a factor in glucosinolate variation and to determine whether such variation might be due to translocation of glucosinolates between the aerial parts of the plant and its roots. Cabbage seedlings were maintained at 20 and 30°C over 2 days and leaves and roots sampled at 02:00 h, 06:00 h, 10:00 h, 14:00 h, 18:00 h and 22:00 h. The glucosinolates 2-propenyl- and 3-methylsulphinylpropyl- with an average of 261 and 167 μmol 100 g⁻¹ DW, respectively, were the two main glucosinolates in the aerial part of the plant whilst in the roots 1-methoxyindol-3-ylmethyl-, 2-phenylethyl- and 3-methylsulphinylpropyl, with 495, 495 and 385 μmol 100 g⁻¹ DW respectively, showed the highest average concentrations. Total and individual glucosinolates in the roots and in the aerial part of the plant showed the highest concentrations in the dark cycle, at 02:00 h and 22:00 h, respectively, whilst the lowest levels were during the light cycle, mainly at 18:00 h. The results suggest that temperature was not a major factor in the short-term variation in glucosinolate levels. Although there was a very high significant difference between the total glucosinolate levels in the aerial part of the plant (581 μmol 100 g⁻¹ DW) and roots (2124 μmol 100 g⁻¹ DW), the results of the present study do not support the concept of translocation between aerial part and roots, suggesting that other factors may be involved.

Key words: glucosinolates, cabbage, temperature, variation

INTRODUCTION

Glucosinolates are naturally occurring compounds in cruciferous plants which, on hydrolysis by the enzyme myrosinase (thioglucoside glucohydrolase; EC 3.2.3.1), liberate, in addition to sulphate ion and glucose, a range of compounds including isothiocyanates, nitriles and thiocyanates. Such compounds demonstrate a broad range of physiological activities including toxic and antinutritional effects when included in animal diets as well as contributing to the desirable sensory properties of Brassica and other cruciferous crops. More recently, glucosinolate-derived compounds have been shown to contribute to the anticarcinogenic effect of Brassica vegetables. The nature and properties of glucosinolates and their breakdown compounds have been described in several reviews (Fenwick et al 1983; Duncan 1991; Rosa et al 1997). It is important to understand the factors which determine the occurrence and levels of glucosinolates not only between species, but also between individual parts of the same plant. Ontogenic variation has also been reported in Brassica napus (Macfarlane Smith and Griffiths 1988; McGregor 1988; Clossais-Besnard and Larher 1991; Fieldsend and Milford 1994a, b) and B oleracea (Rosa et al 1996). Bergmann (1970) reported a decline in indole glucosinolates and 4-hydroxybenzylglucosinolate in developing seedlings of yellow mustard. Based on measurements of
volatile myrosinase hydrolysis products, Cole (1980) observed a substantial decline in levels of aliphatic glucosinolates over the first few days of development in seedlings of turnips, Chinese cabbage, fodder rape (B. campestris L), cauliflower (B. oleracea var. botrytis) and radish (Raphanus sativus L). A thorough understanding of glucosinolate metabolism in plants requires intensive studies of their distribution between plant organs and changes during the various development stages. Major differences in the relative amount of individual glucosinolates have been observed between the different parts of developing rapeseed plants (McGregor 1988), indicating that individual glucosinolates have defined distribution patterns. Furthermore, large differences between seed, leaf and root glucosinolate profiles of several brassicas have been described by Sang et al (1984). In a recent publication (Rosa et al 1994) we observed that, under field conditions, glucosinolates in the leaves of young cabbage plants showed significant variation throughout a single day. Although the data suggested a rapid metabolism of glucosinolates, the causes of this variation are not fully understood.

In the present study we report the results of two experiments designed to elucidate, under different temperature conditions in a controlled environment, the short-term variation in the levels of individual and total glucosinolates in both the aerial parts and the roots of developing seedlings of B. oleracea var. capitata.

**MATERIALS AND METHODS**

Seeds of the pointed white cabbage Brassica oleracea var. capitata cultivar Green Point were sown in a mixture of 1 vol sand (0-02–0.2 mm): 3 vol peat compost (v/v) (Humobact Terreau, Frans Baele SA, France) in polypropylene trays with alveoles of 65 cm$^2$. Seeds were placed at a depth of 1.5 cm and covered with vermiculite. Plants were grown in a growth cabinet (Conviron E15) with a 14 h photoperiod, 07:00 h to 21:00 h, a photosynthetic photon flux density of 480 μmol m$^{-2}$ s$^{-1}$ and a 75% relative humidity. In experiment 1, temperature was maintained at 25°C during the light cycle and 18°C between 21:00 h and 07:00 h. At 14 days after emergence (DAE) the temperature was set to a constant 20°C and after a further 24 h, sampling was at 02:00 h, 06:00 h, 10:00 h, 14:00 h, 18:00 h and 22:00 h over a period of two consecutive days (15 and 16 DAE). Experiment 2 was an extension of experiment 1 with the plants allowed to grow for a further week at conditions of experiment 1. At 21 days after emergence a constant temperature of 30°C was set and after 24 h, samples were taken over 2 days (22 and 23 DAE) at the same intervals described above. In both experiments the aerial parts (mainly leaves) and the roots were analysed separately. Thus, plants were lifted from the vermiculite and the intact aerial part, with 5 to 7 true leaves, was separated from the roots by cutting the stem at the soil level and immediately frozen in liquid nitrogen. The roots were separated from the peat compost by soaking in water and gently washing in a continuous water flow to remove all peat residues without damage. Excess water was removed with absorbent paper and the roots were freeze-dried. Using tissue from five randomised plants, three replicates were obtained for each sampling time. After freeze-drying, samples (c 200 mg) were extracted by addition of boiling 90% methanol (3 ml plus 0-4 μmol benzyl glucosinolate as an internal standard) and maintaining boiling for 10 min, a procedure which simultaneously inactivates myrosinase. After filtration, the residue was re-extracted twice using boiling 70% methanol (3 ml). The extracts were combined to give a total volume of 10 ml and an aliquot (3 ml) was evaporated to dryness and taken up in water (3 ml) of which 2 ml was applied to small columns of DEAE Sephadex A25 and the absorbed glucosinolates desulphated as described by Heaney and Fenwick (1980). Desulphoglucosinolates were eluted with water and analysed using the high-performance liquid chromatography (HPLC) procedure described by Spinks et al (1984). The effect of temperature on glucosinolate concentration in both the leaves and the roots was assessed using an analysis of variance and a correlation analysis was performed between both parts of the plant. All statistical analysis was done using a SuperAnova 1.1 software.

**RESULTS AND DISCUSSION**

The major glucosinolates in the aerial part of the cabbage seedlings were 2-propenyl-, 3-methylsulphinylpropyl-, 2-hydroxybut-3-enyl- and but-3-enyl- with minor amounts of indol-3-ylmethyl- and 4-hydroxyindol-3-ylmethyl-glucosinolates. In the roots 2-phenylethyl-, 1-methoxyindol-3-ylmethyl-, 4-methylthiobutyl-, 2-propenyl- and 3-methylsulphinylpropyl- were the main glucosinolates with minor amounts of 2-hydroxybut-3-enyl-, but-3-enyl-, indol-3-ylmethyl- and 4-hydroxyindol-3-ylmethyl-glucosinolates. In both experiments (Figs 1 and 2), the levels of total glucosinolates in the roots, averaged over all sampling times, were significantly higher ($P < 0.001$) than in the leaves, in agreement with the findings of Josefsson (1967a). The glucosinolates found in this study were qualitatively similar to those reported for heads of white cabbage by Sones et al (1984). At this early stage of development, the levels of indole glucosinolates (particularly 1-methoxyindol-3-ylmethyl-) were significantly higher ($P < 0.001$) in the roots than in the leaves, where aliphatic glucosinolates predominate. The present study confirms the finding of Josefsson (1967b) who reported higher levels of indolyl glucosinolates (measured as thiocyanate ion) in the roots of turnip...
Fig 1. Individual and total glucosinolate variation in the leaves (○) and roots (●) throughout the day at a constant temperature of 20°C during the light (07:00 h to 21:00 h) and dark cycle. When SE bars are not shown, the SE is smaller than the symbols.

Fig 2. Individual and total glucosinolate variation in the leaves (○) and roots (●) throughout the day at a constant temperature of 30°C during the light (07:00 h to 21:00 h) and dark cycle. When SE bars are not shown, the SE is smaller than the symbols.
rapeseed during the growth period. Our results reveal that the major indolyl compound in rapeseed roots is 1-methoxyindol-3-ylmethyl glucosinolate.

In both experiments, the roots were characterised by high levels of 2-phenylethyl-, 4-methylthiobutyl- and 1-methoxyindol-3-ylmethyl glucosinolates. These compounds, which were absent from the leaves, account for much of the difference between total glucosinolate levels in the roots and those observed in leaves. In experiment 1, levels of 3-methylsulphinylpropyl glucosinolate were significantly higher in the roots than in the leaves, whereas in experiment 2 the reverse was true and levels of this compound were greatly reduced in both leaves and roots, probably due to the plants later stage of development. Similarly, apart from 2-hydroxybut-3-enyl glucosinolate, average levels of all other glucosinolates were lower at 30°C than at 20°C probably due the plant being 7 days older, a situation that induces lower glucosinolate levels (Rosa et al. 1996).

In an earlier study (Rosa et al. 1994) we reported that glucosinolates in the leaves of young rapeseed plants grown in the field, showed considerable variation throughout the course of a single day. These experiments show that such variation still occurs under controlled conditions and further demonstrates that the glucosinolates in the roots of the plants are also subject to dramatic variation (Figs 1 and 2).

When temperature was maintained at a constant 20°C (experiment 1) there were significant differences ($P < 0.001$) between sampling times for total and individual glucosinolates in the leaves, although 2-propenyl glucosinolate was the least significant ($P < 0.05$), whilst in the roots only this glucosinolate showed significant variation ($P < 0.05$). At higher temperatures (at a constant 30°C—experiment 2), in the leaves, indol-3-ylmethyl- and 2-phenylethyl glucosinolates did not show any significant differences between sampling times. A similar result was observed for 4-hydroxyindol-3-ylmethyl- and indol-3-ylmethyl glucosinolates in the roots. Higher temperatures induced larger differences, between 1072 mmol 100 g$^{-1}$ DW at 10:00 h to 2489 mmol 100 g$^{-1}$ DW at 02:00 h in roots and between 368 mmol 100 g$^{-1}$ DW at 14:00 h and 910 mmol 100 g$^{-1}$ DW at 02:00 h in the leaves (Fig 2).

The lowest total glucosinolate levels were observed during the first half of the light cycle with a single recovery in the next hours. Even at low temperatures (experiment 1) the lowest total glucosinolate levels in the roots were noted during the light cycle (Fig 1). The trends at a constant 20°C indicate that total glucosinolates in the roots are at their lowest levels during the light period and reach their highest levels during the middle of the dark period (02:00 h). This situation is particularly evident when plants are submitted to higher temperatures, under which conditions leaves follow a similar trend. It would be tempting to conclude that this effect was induced by light or was a consequence of temperature-induced stress, but the increase in levels at 18:00 h (Fig 2) suggests that other factors may be involved. Individual glucosinolates generally followed the trend of totals.

A cursory examination of the results of experiment 1 (20°C constant) (Fig 1) suggests that for some glucosinolates, particularly 2-hydroxybut-3-enyl- and 2-propenyl glucosinolates, an inverse relationship exists between levels in leaves and those in roots possibly indicating a turnover in the glucosinolates between roots and leaves. However, this is not supported by the correlation analysis between both parts of the plant. Furthermore, in experiment 2 at stress temperatures (30°C constant), levels in leaves closely followed levels in roots (Fig 2).

The proposal (Schnug 1991), that glucosinolates constitute a reserve supply of sulphur for use in protein biosynthesis and that this source is mobilised in situations of soil sulphur deficiency or high sulphur demand for growth has been questioned by Fieldsend and Milford (1994a). In the present study the plants were well supplied with sulphur and unless the leaves make short-term demands for sulphur it seems unlikely that this is the reason for observed changes. However, it is possible that a recycling process occurs involving the initial hydrolysis of glucosinolates by myrosinase, as proposed by Schnug (1991), but rather than reassimilation of the liberated sulphate or isothiocyanate sulphur into aminoacids, they could be used in other biological processes. Since, during the light cycle and particularly at stress temperatures, there is a depression in glucosinolate content, it is likely that at this stage the aminoacid precursors of the glucosinolates could be used in other metabolic activities other than for glucosinolate biosynthesis.

Glucosinolates can themselves be mobilised within the plant as observed in rapeseed between pod walls and seed by Bilsborrow et al. (1993). In our study mobilisation of intact glucosinolates is unlikely to occur because entire plants were analysed and no correlation between these two plant parts was observed.

Salisbury and Ross (1992) have suggested that roots always have the ability to synthesise enough auxins for their growth which indicates that tryptophan must be present as the precursor of IAA and of the respective intermediate compounds such as indol-3-ylmethyl glucosinolate. Thus, it is likely that this glucosinolate is synthesised in the roots.

**CONCLUSIONS**

In both experimental regimes, the levels of total glucosinolates were between 3 and 4 times higher in the roots of young rapeseed plants than in the aerial parts, reflecting the high levels of 2-propenyl-, 1-methoxyindol-3-ylmethyl- and 2-phenylethyl-glucosinolates. Indole glu-
cosinolates were higher in the roots than in the aerial part of the plant, where aliphatic glucosinolates predominate.

This study demonstrates the variable nature of glucosinolate levels in leaf tissue throughout the course of a single day as reported earlier (Rosa et al. 1994). It is further shown that this variability occurs not only in the aerial parts but also in the roots of the plant. When plants were kept at 30°C (experiment 2), glucosinolate levels dropped significantly from their highest levels at 02:00 h before peaking again at 18:00 h. Lower temperatures (20°C constant) induced smaller variations throughout the day, however, with the same tendency to peak at 02:00 h as shown at higher temperatures. Thus, factors other than temperature may be involved in glucosinolate variation within the plant and further studies are underway to clarify this situation.

REFERENCES

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