VARIETAL DIFFERENCES IN SULPHUR UPTAKE AND UTILIZATION IN RELATION TO GLUCOSINOLATE ACCUMULATION IN OILSEED RAPE

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ABSTRACT: This paper compares the uptake and utilization of S in relation to glucosinolate accumulation in seeds in a double-low variety Cobra and a single-low variety Bienvenu. There were little differences between the two varieties in S uptake and distribution during vegetative growth. Major differences were found after pod formation. Bienvenu had much higher concentrations of glucosinolates in both pod walls and seeds, whereas Cobra accumulated a large amount of S, mainly as sulphate, in the pod walls. The results support the idea that pod walls are the major site of the biosynthesis of glucosinolates present in seeds and a block in the pathway of glucosinolate biosynthesis in the pod walls is likely to be responsible for the low glucosinolate concentration in the double-low rapeseed.

INTRODUCTION

Oilseed rape crops require a relatively large amount of S, because S is needed in the biosynthesis of both proteins and glucosinolates. Cultivars of oilseed rape grown in the EC countries are now of the double-low type. However, the biochemical and physiological background of the low glucosinolate trait in double-low rapeseed has not been fully understood. Also, the differences between double- and single-low varieties in S nutrition remain to be elucidated. This paper compares double- with single-low varieties in S uptake and distribution in relation to glucosinolate accumulation in seeds.

EXPERIMENTAL

Field experiments were conducted at Cockle Park Farm, Northumberland, UK during 1988-92 to compare S uptake, distribution and glucosinolate accumulation of a double-low variety Cobra and a single-low variety Bienvenu. Young developing pods were used in an incubation experiment to investigate the uptake and distribution of sinigrin. Glucosinolates were determined by HPLC. Total S in plant material was measured by X-ray fluorescence. Sulphate-S in leaves was determined by the HI-reduction method and in pod walls by ion chromatography. The details of these experiments have been presented previously (Bilsborrow *et al.* 1993; Zhao *et al.* 1993a,b).

RESULTS AND DISCUSSION

Both Cobra and Bienvenu produced similar amounts of dry matter of about 20 t/ha at maturity. There were no significant differences between the two varieties in total S uptake during the entire growth period and at maturity total S uptake amounted to approximately 90 kg/ha. The concentrations of S in roots, stems and leaves were very similar in Cobra and Bienvenu. During the early growth stage up to rosette, sulphate-S accounted for 15-45% of the total S in leaves and the percentages at each sampling date were almost identical in the two varieties.

Major differences between Cobra and Bienvenu were found in the pods. In Cobra, there

was a large and progressive build-up of S in the pod walls, whereas in Bienvenu the concentration of S in the pod walls changed little (Fig. 1a, b). In contrast, the concentrations of both total S and glucosinolates in the seeds were much smaller in Cobra than in Bienvenu (Fig. 1c, d). The accumulation of S in the pod walls of Cobra was mainly in the form of sulphate-S. This was further confirmed in a separate experiment using freeze-dried materials of pod walls and an extraction with 10 mM ascorbic acid to suppress myrosinase activity completely (Zhao *et al.* 1993b). The concentrations of glucosinolates in the pod walls were much higher in Bienvenu than in Cobra, and in both varieties they decreased during the active phase of seed growth (Fig. 1e). The decreases corresponded with the increases of glucosinolate concentrations in the seeds.

In an incubation study, young developing pods of Cobra and Bienvenu were fed with a solution containing sinigrin, a glucosinolate which is absent in rapeseed. Sinigrin concentrations in pod walls increased during the first 4 h, and decreased rapidly in the next 16 h when the exogenous supply of sinigrin was withdrawn. In contrast, the concentrations of sinigrin in seeds increased rapidly during the entire incubation period, even after the withdrawal of the exogenous supply. The results suggest that sinigrin was actively transported from pod walls to seeds in both varieties.

It has been suggested that double-low varieties are more susceptible to S deficiency because of a defect in glucosinolate biosynthesis in vegetative tissues (Schnug and Haneklaus 1993). These authors suggest that S-containing intermediates accumulate in the double-low plants, which, unlike glucosinolates, cannot be re-utilized when S shortage occurs. This hypothesis is not supported by the results obtained in the present study, which show that the double-low Cobra differs little from the single-low Bienvenu in the S uptake and utilization during the vegetative

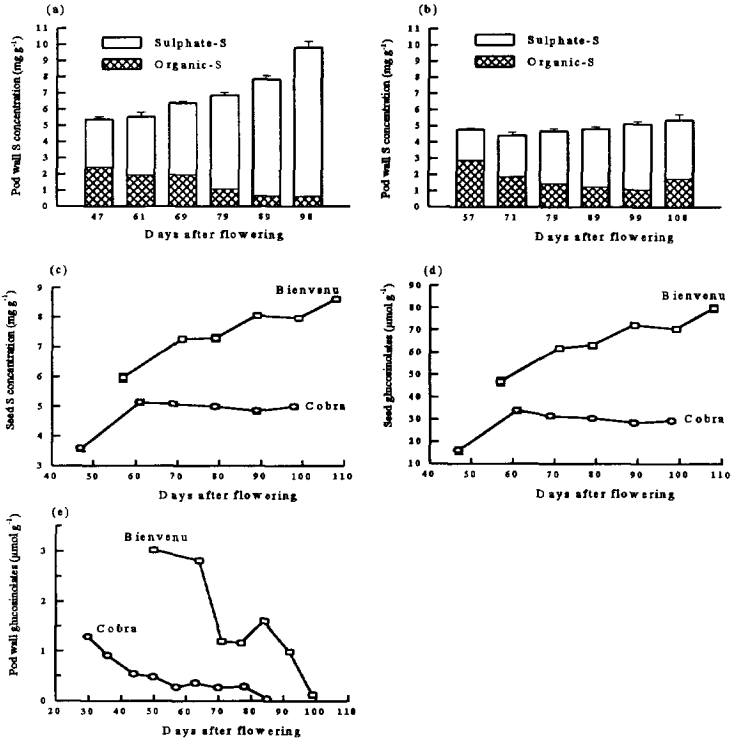

Fig. 1 Changes of the concentrations of total and sulphate-S in the pod walls of Cobra (a) and Bienvenu (b), total S in seeds (c), glucosinolates in seeds (d) and glucosinolates in pod walls (e).

growth. Many other studies have shown that the concentrations of glucosinolates in vegetative tissues are within similar ranges in various double- and single-low varieties (Milford *et al.* 198 9; Wynne Griffiths *et al.* 1994). The activities of several enzymes involved in the initial steps of glucosinolate biosynthesis are similar in the leaves of Cobra and Bienvenu (Wallsgrove *et al.* 1993). Therefore, there is no evidence that double-low varieties have a defect in glucosinolate biosynthesis in the vegetative tissues. Also, the role of glucosinolates as S storage compounds in vegetative tissues would be very limited, because glucosinolate-S accounts for less than 10% of total S in the leaves of both single- and double-low varieties (Fieldsend and Milford 1994). The amount of glucosinolate-S in leaves is much smaller than that of sulphate-S found in the present and other studies (Withers and O'Donnell 1994). Based on these results, the hypothesis of Schnug and Haneklaus (1993) is rejected. The suggestion that double-low varieties are more sensitive to S deficiency than single-low varieties has not been confirmed unequivocally. Walker and Booth (1994) found that the double-low variety Tapidor was more responsive to S fertilization than the single-low variety Rafal in a field trial, but the other double-low variety Cobra was as responsive as Rafal. It is emphasised that factors other than glucosinolate concentration, such as root morphology and the rate of plant growth, can contribute to the varietal differences in the susceptibility to S deficiency.

The results from this study support the idea that pod walls are the major site of the biosynthesis of glucosinolates present in seeds (MaGrath and Mithen 1993; Gijzen *et al.* 1994). A block in the pathway of glucosinolate biosynthesis in the pod walls of double-low varieties would result in low concentrations of glucosinolates in both pod walls and seeds, and a large accumulation of S, mainly as sulphate, in the pod walls. The build-up of sulphate is a certain consequence if such a metabolic block exists, because the incorporation of sulphate into glucosinolate molecules takes place at the very final stage of the biosynthesis. Accumulation of S-containing intermediates, as suggested by Schnug and Haneklaus (1993), is likely to be insignificant, since a feedback mechanism would suppress the synthetic reactions towards these compounds. Recently, Dawson *et al.* (1993) have shown that the activities of the enzymes responsible for the formation of aldoxime, a key intermediate of glucosinolate biosynthesis, are inhibited significantly by the product itself, indicating the existence of a feedback regulation.

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