PHYSIOLOGY OF INDOLE GLUCOSINOLATE INCORPORATION IN RAPESEED (BRASSICA NAPUS L.)

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INTRODUCTION

Glucosinolate (GSL) content in 00-rapeseed has been reduced to about 10 % of the older 0-varieties by using the low-GSL cultivar 'Bronowski' for breeding. This reduction was achieved by eliminating most of the alkenyl GSLs. Up to now no real reduction of the indole GSLs could be detected and their percentual volume is still increasing to more than 50 % of all GSLs. So genotypes with lower indole GSL content are receiving particular attention of the breeders.

In our institute different amounts of indole glucosinolates (GSLs) in rapeseed lines (Fig. 1) were detected by screening large quantities of breeding material (Kräling et al. 1990). Three of those lines with differing and stable indole GSL-profiles are used in feeding experiments to examine the incorporation of these indolic compounds into the seed. 4-hydroxy-glucobrassicin (4OH), the main indole GSL in the seed, is not present in green plant parts. So it is discussed, whether the biosynthesis of this compound occurs in the pod, followed by a transport into the seed, or if the biosynthesis is situated in the seed itself.

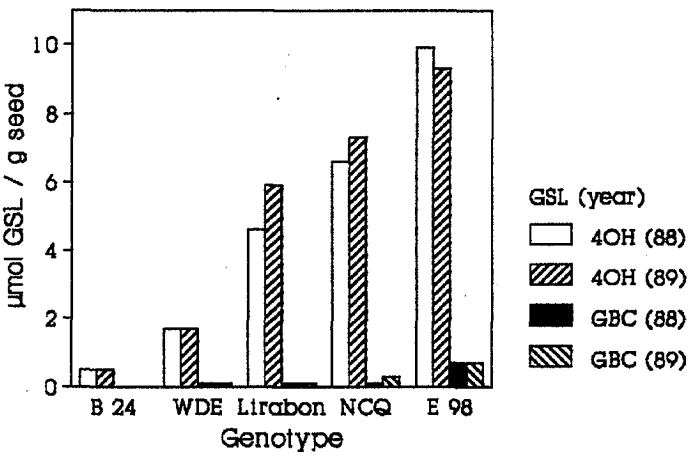

Fig. 1. Range of indole glucosinolate content in the examined genotypes

MATERIAL AND METHODS

Plants

Embryos of line B24 (< 0.5 µmol indole GSL / g seed), line E98 (ca. 10.0 µmol indole GSL / g seed) and cultivar 'Librador' (ca. 5.5 µmol indole GSL / g seed) were aseptically excised from seeds 24 days after selfpollination by hand and cultured in 5 ml MS-medium (440 mg / 100 ml; pH 5.8), containing 3 % sucrose and 0.7 % agarose. With exception of the control samples, 2.5 µmol tryptophan (Trp), sinigrin (SIN), glucobrassicin (GBC) or 4OH were added. One sample consisted of 10 embryos, cultured for 2 to 5 days in petri dishes (5 cm diameter) under fluorescent lights at room temperature. Each experiment was repeated twice.

Germinating seeds of the lines, mentioned above, were cultured for 2 to 4 days in MS-medium without and with 2.5 µmol GBC added.

Analytical method

Embryos or germinated seeds were washed twice with 2 ml of distilled water and placed in 10 ml-glass potters heated in a waterbath (80°C). 1 ml hot 70 % aqueous methanol and 50 µl glucotropaeolin (GTL; internal standard, 5 mmol/l) were added and after 5 minutes the embryos were pottered twice and left in the waterbath for further 5 minutes. The extract was collected and combined with 1 more ml 70 % methanol, used for rinsing the potter. Samples were mixed and centrifuged (2500 g) for 10 min. GSLs were desulfated with sulfatase (Sigma) on a DEAE-Sephadex column and analysed by HPLC, as published before (Kräling et al. 1990).

RESULTS

Embryos

Feeding of Trp did not result in any measurable GSL. The uptake of Trp itself was not determined. After the addition of GSLs these compounds were accumulated in the embryo. Furtheron, 4OH was detected when GBC was added to the medium (Fig.2).

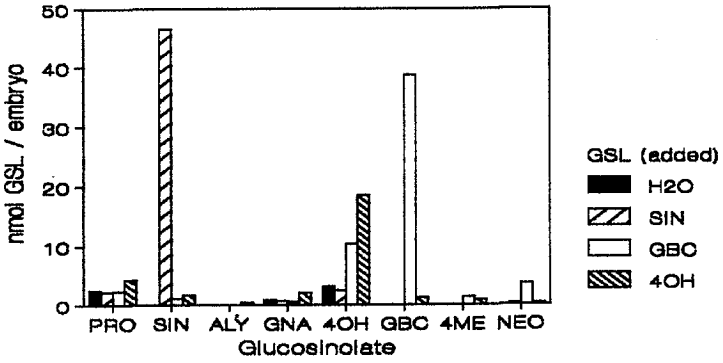

Fig. 2. GSL pattern of embryos of cv. 'Librador' after 4 days in GSL containing medium

The amount of formation of 4OH was dependent on the genotype. About 50 nmol indole GSL was found after culturing of embryos in GSL containing medium. Whereas in B 24 and in 'Librador' about 10 nmol of GBC were transformed into 4OH, in E 89 about 20 nmol of 4OH could be detected after 4 days (Fig. 3).

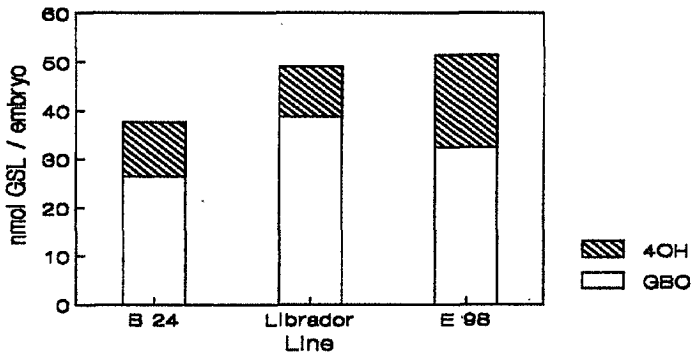

Fig. 3. Uptake of glucobrassicin (GBC) and biosynthesis of 4-hydroxy-glucobrassicin (4OH) in different genotypes

Germinating seeds

Uptake of GBC in germinating seeds was not as successful as in embryos. Only half of the above mentioned rate could be examined. In 'Librador'and E 98 formation of 4OH was detectable, wheras in B 24 no difference in the amount of 4OH was measured compared with the control samples.

DISCUSSION

All added GSLs were accumulated by the embryo. The lower uptake of 4OH, as compared with GBC and SIN, could be explained by the instability of this GSL in the medium (Truscott 1989), demonstrated by the greyish colour of the medium in this case, but not by a lower accumulation rate. The uptake of GSLs, independent of the side chain, was earlier shown for alkenyl- and benzyl-GSLs by Gijzen et al. (1989).

When the formation of 4OH in the embryo is compared with the 4OH amount in the seed in situ, similarities are found for 'Librador' and E 98. The enzyme activity in E 98 seems to be 200 % of that in 'Librador'. This would explain the high amount of 4OH in the seed of E 89. If 4OH is synthesized in the seed, in B 24 a precursor of 4OH may be missing in the embryo due to a biosynthetic block, because enzyme activity could be detected in the same range as in the 00-cultivar 'Librador'.

Further investigations are necessary, since a synthesis of 4OH is possible also in germinating seeds when GBC is added, although in situ 4OH is decreasing, while GBC increases in the first days of germination (McGregor 1988).

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