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Genetics of aliphatic glucosinolates. II. Hydroxylation of alkenyl glucosinolates in *Brassica napus*

I. PARKIN, R. MAGRATH, D. KEITH, A. SHARPE, R. MITHEN* & D. LYDIATE

Brassica and Oilseeds Research Department, John Innes Centre, Institute of Plant Science Research, Colney Lane, Norwich NR4 7UJ, U.K.

The genetic regulation of hydroxylation of butenyl and pentenyl glucosinolates in *Brassica napus* is investigated by studying the segregation of the degree of hydroxylation in seeds and leaves of recombinant lines derived from crosses between oilseed rape cultivars and synthetic *B. napus* lines. It is shown that two loci regulate hydroxylation in both leaves and seeds. Alleles at a locus on linkage group 13 (*Gsl-oh-C*) have a major effect while alleles at a homoeologous locus on group 3 (*Gsl-oh-A*) have a minor effect. The implication of these results for developing improved cultivars of oilseed rape is discussed.

Keywords: *Brassica*, glucosinolates, gene mapping, 2-hydroxy-3-butenyl glucosinolate, restriction fragment length polymorphisms (RFLP).

Introduction

Glucosinolates are thioglycosides which occur in members of the Capparales which include economically important *Brassica* oilseed and vegetable crops. The molecule is composed of two parts, a common glycone moiety and a variable aglycone side chain derived from α-amino acids (Fig. 1). Following cellular disruption, glucosinolates undergo hydrolysis to produce an array of products dependent upon the nature of the side chain. Alkenyl glucosinolates, which are the predominant glucosinolates in the leaves and seeds of oilseed rape, produce isothiocyanates which determine the palatability of the leaves of oilseed rape to vertebrate pests such as pigeons and rabbits (Mithen, 1992), and the palatability of rapeseed meal to livestock. If the alkenyl glucosinolates possess a β-hydroxyl group, the isothiocyanates spontaneously cyclize to produce oxazolidine-2-thiones (Fig. 1) which are goitrogenic when ingested by livestock (Fenwick *et al.*, 1983; Poulton & Møller, 1993). The antinutritional effects of glucosinolates have led plant breeders to develop low glucosinolate oilseed rape cultivars. However, within both high and low glucosinolate cultivars, 2-hydroxy-3-butenyl glucosinolate represents the most abundant glucosinolate in rapeseed, accounting for up to 80 per cent of total glucosinolates (Fig. 2). In order to enhance further the quality of rapeseed, it would be desirable to prevent the hydroxylation of alkenyl glucosinolates which would eliminate the major goitrogenic compound. Moreover, the prevention of hydroxylation of alkenyl glucosinolates may also result in a decrease in the palatability of leaf tissue to pests due to the concomitant rise in butenyl glucosinolate (Mithen, 1992).

All oilseed rape cultivars and other natural forms of *B. napus* (genome AACC) have high levels of hydroxyalkenyl glucosinolates in their leaves and seeds. In an earlier study, the development of synthetic *B. napus* lines from the interspecific hybridization of *B. rapa* and *B. oleracea* (the A and C genome donors, respectively) was described (Magrath *et al.*, 1993). These lines did not produce significant amounts of hydroxyalkenyl glucosinolates. It was shown that the hydroxylation of alkenyl glucosinolates in leaves is regulated by alleles at two unlinked loci, one of which must be in the A genome and one in the C genome. In leaves, functional alleles at one of the loci result in approximately 40 per cent hydroxylation of butenyl glucosinolates while those at the other locus result in approximately 20 per cent hydroxylation, and both act in an additive manner so that in oilseed rape cultivars which are homozygous for the functional alleles at both loci there is approximately 60 per cent hydroxylation of butenyl glucosinolates (Magrath *et al.*, 1993). The hydroxylation of butenyl glucosinolates in seeds is largely determined by a single locus. The hydroxylation of pentenyl glucosinolates is proportional to the hydroxylation of butenyl glucosinolates and it is likely that it is regulated

*Correspondence.

by the same genes (Magrath *et al.*, 1993). It would be desirable to introgress null alleles for hydroxylation into *B. napus* cultivars from synthetic *B. napus* lines. However, as functional alleles at each of the hydroxylation loci are dominant, individuals which are heterozygous at the hydroxylation loci can only be selected in backcrossing programmes with the use of test crosses. In the present paper we provide further evidence for

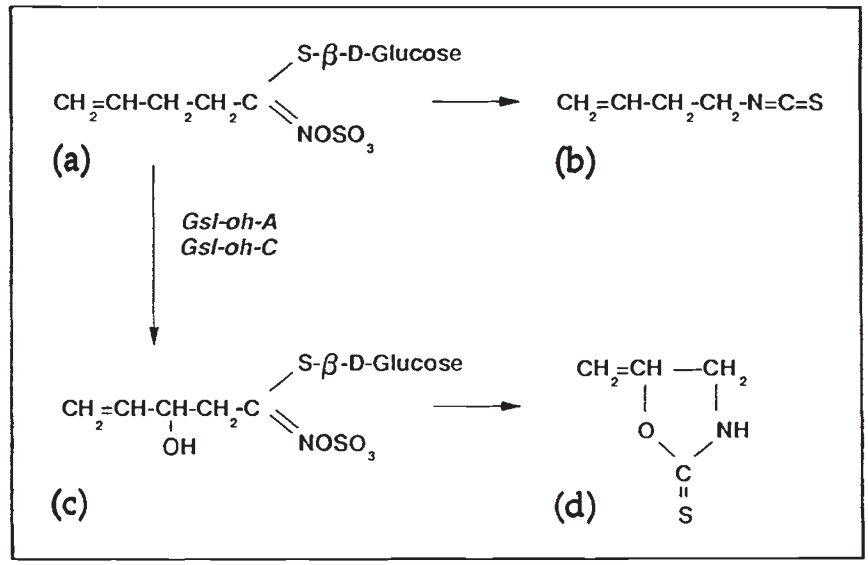

Fig. 1 Hydrolysis products of butenyl and 2-hydroxy-3-butenyl glucosinolates, and the conversion of butenyl to 2-hydroxy-3-butenyl glucosinolate. Further details of the biosynthetic pathway of aliphatic glucosinolates can be found in Magrath *et al.* (1994). (a) Butenyl glucosinolate, (b) butenyl isothiocyanate, (c) 2-hydroxy-3-butenyl glucosinolate, (d) 5-vinyloxazolidine-2-thione.

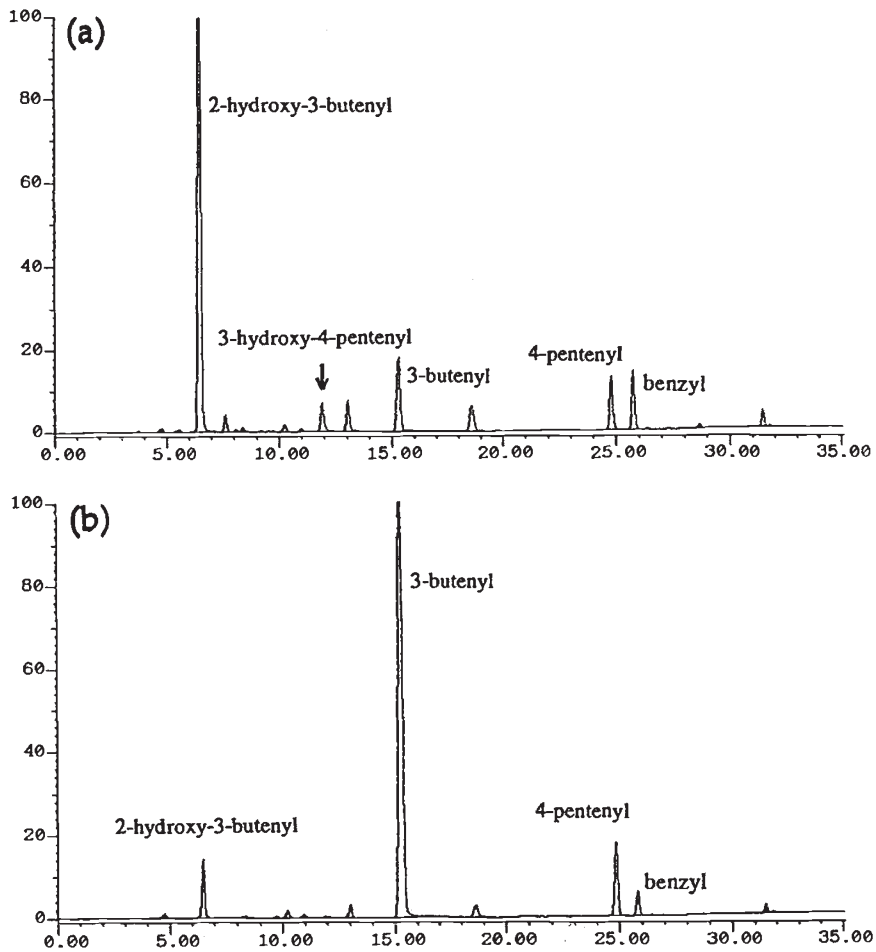

Fig. 2 HPLC chromatogram of desulphoglucosinolates extracted from seeds of a natural *B. napus* line (a) and a synthetic *B. napus* line (b).

the genetic regulation of hydroxylation of alkenyl glucosinolates in leaves and seeds, and position the loci which regulate hydroxylation on a *B. napus* RFLP map.

Materials and methods

The RFLP mapping studies were undertaken on recombinant homozygous lines derived by the culture of microspores from F_1 hybrids between natural and synthetic *B. napus* lines. Two populations derived from different parents were studied. Firstly, leaf glucosinolates were extracted and analysed in 54 homozygous recombinant lines derived by microspore culture of an F_1 hybrid from a cross between *B. napus* cv. Cobra and a synthetic *B. napus* line, as described previously (Magrath *et al.*, 1993, 1994). Secondly, seed glucosinolates were extracted and analysed from 32 homozygous recombinant lines derived by microspore culture of an F_1 hybrid from a cross between a winter *B. napus* breeding line and a synthetic *B. napus* line derived from the hybridization of *B. rapa* subsp. *chinenesis* and *B. oleracea* var. *alboglabra*. This synthetic line was obtained from Dr T. Hodgkin, formerly of the Scottish Crops Research Institute. Microspore culture of F_1 hybrids, DNA extraction and restriction, Southern blotting and hybridization were as described previously (Magrath *et al.*, 1994). Fifty recombinant lines from the second cross (including the 32 lines from which seed glucosinolates were analysed) were probed with 168 genomic probes which detected over 400 RFLP loci. Full details of the RFLP map derived from these lines will be published separately (Parkin *et al.*, in preparation). Extraction of glucosinolates, conversion to desulphoglucosinolates, HPLC analysis and calculation of the percentage hydroxylation of butenyl and pentenyl glucosinolates were as described previously (Magrath *et al.*, 1993). Recombination analysis and RFLP mapping were undertaken with the assistance of the MAPMAKER program, as described previously (Magrath *et al.*, 1994).

Results

Cross 1: Leaf glucosinolate profiles

In the leaves of *B. napus* cv. Cobra there was approximately 60 per cent hydroxylation of butenyl glucosinolates while there was less than 1 per cent hydroxylation of butenyl glucosinolates in the synthetic line (Magrath *et al.*, 1993). In the 54 recombinant lines, there was segregation of the degree of hydroxylation of butenyl glucosinolates (Fig. 3). The segregation agrees with predictions made from studies on F_2 and backcross populations (Magrath *et al.*, 1993). Firstly, there was segregation into two major classes: one class of 33 genotypes had between 0 and 26 per cent hydroxylation (class 1) and the second class of 21 genotypes had greater than 40 per cent hydroxylation (class 2, Fig. 3). This distribution is due to the segregation of alleles at the first of the two hydroxylation loci. Recombination analysis positioned this major locus 20 cM below RFLP locus *pN22b* on linkage group 13 (Fig. 4). This linkage group is in the part of the *B. napus* genome which is homologous to the *Brassica* Cgenome; thus this hydroxylation locus is designated *Gsl-oh-C*. Secondly, there is segregation within each of the two major classes. Within the first class, 11 individuals had less than 1 per cent hydroxylation (class 3) while the remaining 22 individuals had between 9 and 20 per cent hydroxylation (class 4, Fig. 3). This is due to segregation at the second minor hydroxylation locus, which recombination analyses positioned 15 cM from RFLP locus *pN22a* on linkage group 3. This linkage group is homoeologous to group 13 and is within the part of the *B. napus* genome which is homologous to the *Brassica* A genome. The relative positions of the two hydroxylation loci are analogous to the *Gsl-elong-A* and *Gsl-elong-C* loci (Magrath *et al.*, 1994).

Cross 2: Seed glucosinolate profiles

2-Hydroxy-3-butenyl glucosinolate was the dominant glucosinolate in the seeds of the winter *B. napus* breeding line, but represented only a small fraction of the total glucosinolates in the seeds of the synthetic line (Fig. 2). In the recombinant lines, the degree of hydroxylation segregated into two distinct classes. One

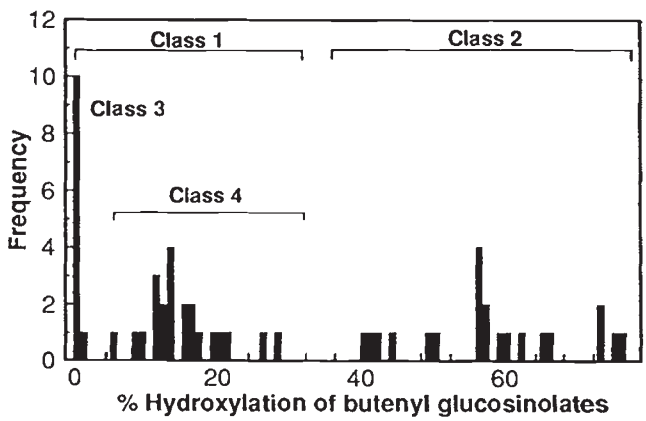

Fig. 3 Segregation of the percentage hydroxylation of butenyl glucosinolates in leaves of recombinant homozygous lines from cross 1.

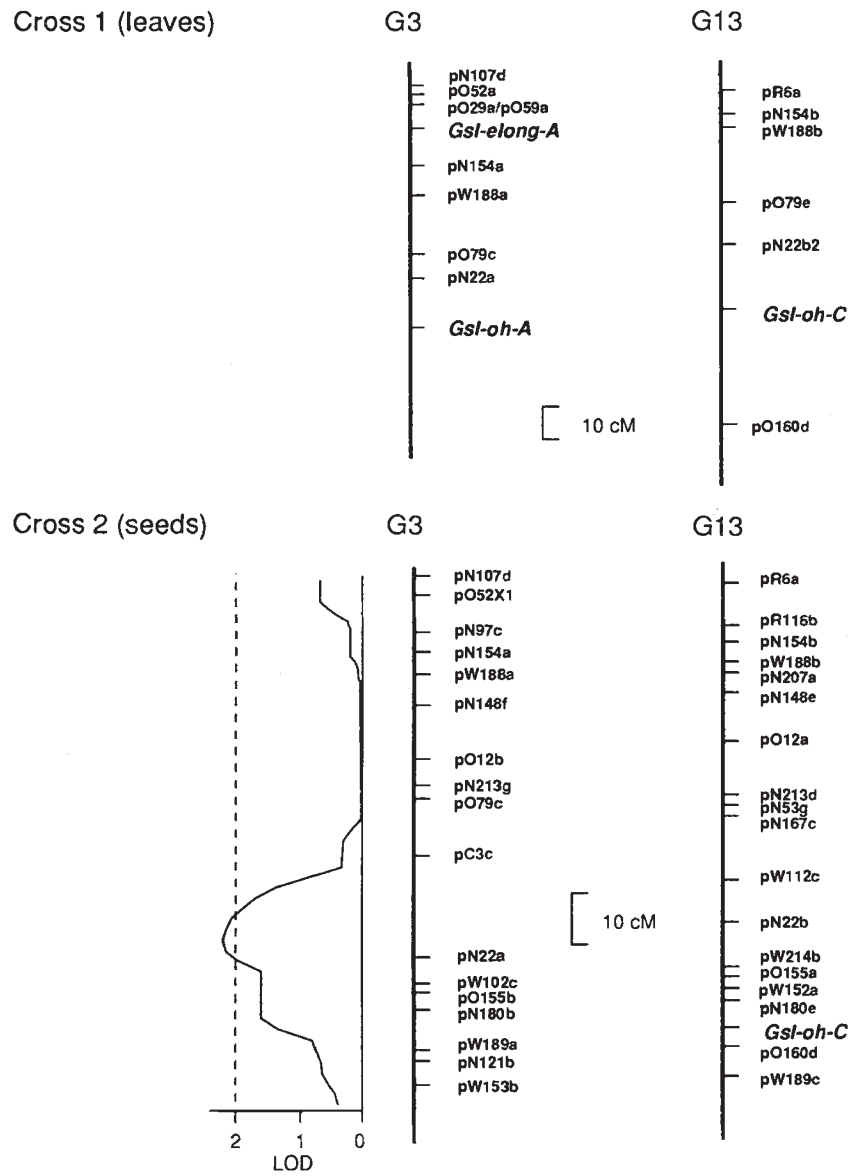

Fig. 4 Linkage maps showing the position of the *Gsl-oh-C* and *Gsl-oh-A* loci on groups 3 and 13. The order of the RFLP loci on the linkage groups from the two crosses is conserved, although distances between loci vary. A region on group 3 in cross 2 has a small quantitative effect on the degree of hydroxylation due to the *Gsl-oh-A* locus mapped in cross 1.

class of 17 individuals had low levels of hydroxylation of both butenyl and pentenyl glucosinolates, while 15 individuals had high levels of hydroxylation (Fig. 5). The two classes corresponded to the parental phenotypes. There were no discrete groups within either of the two classes. The 1:1 segregation ratio is consistent with the hypothesis that alleles at a single locus have a major effect in regulating hydroxylation within seeds. Recombination analyses positioned this locus between the RFLP loci *pN180e* and *pO160d* on group 13. Analysis with MAPMAKER QTL of the variation within the 17 individuals which had low levels of hydroxylation revealed that part of linkage group 3 had a small but significant effect on the extent of hydroxylation. This region coincides with the region in which the *Gsl-oh-A* locus had been mapped in cross 1 (Fig. 4).

Discussion

In a previous report it was shown that two loci regulate hydroxylation of leaf alkenyl glucosinolates and that alleles at these two loci have different efficiencies at adding hydroxyl groups to alkenyl glucosinolates (Magrath *et al.*, 1993). This hypothesis has been supported by the results reported in this paper. The locus having the major effect is in part of the *B. napus* genome homologous to the *Brassica* C genome. Alleles

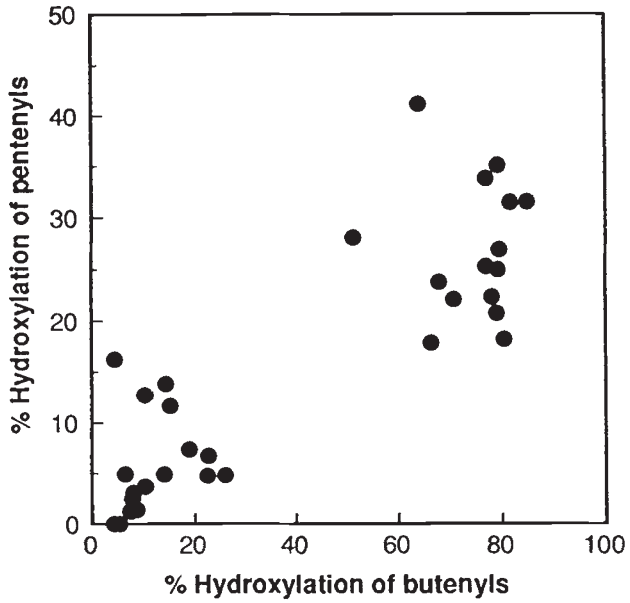

Fig. 5 Segregation of hydroxylation of butenyl and pentenyl glucosinolates in the seeds of recombinant homozygous lines of cross 2.

at this locus are also largely responsible for the hydroxylation of seed alkenyl glucosinolates. A homoeologous locus on group 3 has a minor effect at regulating the hydroxylation of leaf and, to a lesser extent, seed glucosinolates. Introgression of null alleles at the *Gsl-oh-C* locus into *B. napus* breeding lines will improve the quality of meal, and may lead to reduced damage from unspecialized vertebrate and invertebrate pests. The RFLP loci which are linked to the *Gsl-oh-C* locus provide co-dominant molecular markers through which individuals which are heterozygous at the *Gsl-oh-C* locus can be identified in backcross populations, despite having high levels of 2-hydroxy-3-butenyl glucosinolate.

The linkage maps produced from the two crosses were similar despite the contrasting parents used in both crosses. While it is likely that the two oilseed rape lines were genetically similar, the two synthetic *B. napus* lines had been developed from genetically diverse diploid accessions. In each cross the orders of the RFLP loci are conserved although there was considerable difference in the genetic distances between loci.

It has been shown that there is no *de novo* aliphatic glucosinolate biosynthesis in seeds, and that all aliphatic glucosinolates in seeds are derived from the maternal pod tissue (Magrath & Mithen, 1993). The glucosinolate profile of seeds is identical to that of pods but differs from that of leaves in that there is a lower proportion of pentenyl glucosinolates (regulated by the *Gsl-elong-A* locus, Magrath *et al.*, 1994), and that the *Gsl-oh-A* locus does not appear to be important in regulating hydroxylation. Thus both the *Gsl-elong-A* and *Gsl-oh-A* loci appear to be developmentally regulated with reduced expression of alleles at these loci in pod tissue.

The genetic basis of aliphatic glucosinolate biosynthesis in *B. napus* has been used to deduce the glucosinolate profile of the C genome donor (Magrath *et al.*, 1993). From the present study, it has been shown that the major locus determining hydroxylation of alkenyl glucosinolates is within the C genome. The C genome donor must have had functional alleles at this locus which resulted in at least 40 per cent hydroxylation of butenyl glucosinolates in leaves. Thus the leaf aliphatic glucosinolate content of the C genome donor must have comprised 60 per cent butenyl glucosinolates and 40 per cent hydroxybutenyl glucosinolates, the donor having null alleles at the *Gsl-pro* locus and functional alleles at the *Gsl-alk* locus, as explained previously (Magrath *et al.*, 1993, 1994). Potential C genome taxa which have this profile will be described in a subsequent publication.

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