A candidate gene approach identified loci involved in the glucosinolate biosynthesis in mustard (*Brassica juncea*)

Lionneton E* [1,2], Aubert G [1], Ochatt S [1]
[1]INRA, URGAP, BP 86510, 21065 Dijon cedex, France, Eric.Lionneton@epoisses.inra.fr
[2]Fédération des Industries Condimentaires de France, 8 rue d'Isly, 75008 Paris

ABSTRACT

Brassica juncea (2n = 36, genome AABB) is grown in many countries (India, China, and Canada) for oil production. In Burgundy, brown mustard is used for the production of the condiment "Moutarde de Dijon", because of the high sinigrin level in the seeds, responsible for its flavour. The glucosinolate (GSL) composition of *B. juncea* followed two main patterns characterised by high sinigrin (2-propenyl GSL, SIN) and high gluconapin (3-butenyl GSL, GNA), respectively. In these experiments, an AFLP-based genetic linkage map of *B. juncea* was constructed with an F_1-derived doubled haploid population produced by microspore culture. Four QTLs, which regulate the SIN content in *B. juncea* seed, and two QTLs involved in the GNA content were mapped using composite interval mapping. Two of the QTLs for SIN were associated to the QTLs for GNA. A candidate gene approach was developed to identify genes or markers linked to the QTLs involved in sinigrin and gluconapine content. Oligonucleotide primers were designed from the *IPMS-At1* and *IPMS-At2* gene sequences identified as candidate to regulate glucosinolate side chain elongation and located on chromosome 5 of *Arabidopsis thaliana*. Polymorphisms were detected with these primers in the DH population studied and some of the candidate genes were localised, as related to previously mapped QTLs for SIN and GNA. Against this background, and based on previous knowledge about candidate genes, a hypothetical genetic model for the aliphatic GSL biosynthesis in *B. juncea* is proposed.

Key words : *Brassica juncea*, glucosinolates, genetic control, *IPMS* homologous genes

INTRODUCTION

In Burgundy, brown mustard is used for the production of the condiment "Moutarde de Dijon" because of the high sinigrin level in the seeds, responsible for the savour of the condiment. A breeding program was developed to improve many characters, particularly the level of sinigrin in the seeds.

The objective of this study is to better understand the genetic determinism of glucosinolates in condiment *B. juncea* seeds and to identify molecular markers linked to their content. Thus, we decided to map QTL involved in the control of the sinigrin and gluconapin seed content. As, the applicability of consensus PCR primers across species and genera has been demonstrated by using consensus primers between *Arabidopsis thaliana* and *Brassica napus* (Brunel et al. 1999), we developed a candidate gene approach from known gene sequences in *Arabidopsis* controlling the glucosinolate pathway.

The preliminary results of relationships between molecular markers and QTL for sinigrin and gluconapin to develop a marker-assisted selection are discussed.

MATERIAL AND METHODS

A population of doubled haploid (DH) lines was produced from a F1 plant of a cross between BJ-99, an oriental type with yellow seeds containing both butenyl and propenyl glucosinolates and BJ-70, an Indian type with brown seeds that contains only propenyl glucosinolates. The DH population was obtained via microspore culture according to the procedure described by Lionneton et al. (2001) and was evaluated over two years (2001 and 2002) in spring field plots sown at the INRA experimental field at Dijon. Sinigrin (SIN), gluconapin (GNA) and total glucosinolate (GSL) contents of the parents and DH plants were directly determined by near infra red spectrometry (NIRS Foss sytem; WinISI v1.04 software) on samples of 5-10 g seeds.

PCR primers corresponding to genes involved in the glucosinolate pathway were designed from sequences of candidate genes encoding 2-isopropylmalate synthase (*IPMS*),

which are at the *GSL-ELONG* locus, a locus controlling glucosinolate side chain elongation, mapped on the chromosome V from *Arabidopsis thaliana* (Magrath et al. 1994; Campos et al. 2000). Specific primers were designed from the *Arabidopsis GSL-ELONG* region, with the GeneBank accessions AB006708 for *IPMS-At1* and AB0026660 for *IPMS-At2*. Genetic mapping was carried out by looking for polymorphism in the DH population. Loci were mapped with MAPMAKER/EXP v3.0 on the AFLP genetic linkage map constructed by (Lionneton et al. 2002). PCR products were directly sequenced by MWG-BIOTECH (Ebersberg, Germany). QTL detection was performed by composite interval mapping using QTL Cartographer software.

RESULTS

The PCR amplification of specific primers for *IPMS* genes revealed polymorphisms between the two parental lines. The PCR products were directly sequenced and the partial sequences shown significant homologies with the *IPMS* genes described in *Arabidopsis,* these loci were named *IPMS-Bj*. Three *IPMS* homologue genes were mapped on the *B. juncea* map. An *IPMS-Bj*1 gene was located on LG15 and two *IPMS-Bj*2 genes were mapped on LG6 and LG12.

Two major QTLs, controlling the content of SIN and GNA level in seeds, were detected for both years on linkage groups LG12 and LG14 (Table 1). The QTL on LG12 was located near to the *IPMS-Bj*2 locus. The highest levels of SIN were associated to the alleles from BJ-99 and the highest levels of GNA were associated to the alleles from BJ-70 at the two QTLs. Two minor QTLs were also detected, on LG6 for the control of SIN and on LG1 for GNA. Two QTLs, on LG 1 and LG 7, were detected for the total seed content of GSL, with opposite allelic effect. In 2001, a major QTL was detected on LG15 at the *IPMS-Bj*1 locus, and in 2002 another QTL was also mapped on LG6, co-localised with the QTL for SIN, both were affected by the alleles from BJ-70 (Table 1).

Table 1. QTL detected with QTLCartographer for sinigrin, gluconapin and total seed glucosinolates measured in 2001 and 2002.

Trait	Year	Linkage group	Marker	Confidence interval [a]	LOD	R^2 [b]	a [c]
SIN	2001	LG 12	E2M2_10	E2M2_10	3.29	8.6	-10.9
		LG 14	E4M1_2	E6M2_13-E4M7_10	3.65	10.0	-11.6
	2002	LG 6	E4M8_8	E4M8_8-E1M7_14	2.44	7.7	8.8
		LG 12	E2M2_10	E2M2_10	6.13	18.4	-13.4
		LG 14	E6M2_13	E6M2_13- E4M1_2	3.44	10.4	-10.2
NAP	2001	LG 12	E2M2_10	E2M2_10	4.34	15.4	11.4
		LG 14	E4M1_2	E6M2_13- E4M1_2	4.00	14.3	11.2
	2002	LG 1	E1M6_15	E4M7_12-E7M4_5	2.69	7.3	7.7
		LG 12	E2M2_10	E2M2_10- E6M4_14a	4.82	13.5	10.2
		LG 14	E6M2_13	E6M2_13- E4M1_2	3.81	10.3	9.0
GSL	2001	LG 1	E4M1_13	E4M1_13-D08_900	4.15	10.1	5.1
		LG 7	E6M8_8	E6M8_7-E1M2_7	2.53	5.9	-3.8
		LG 15	*IPMS-Bj1*	*IPMS-Bj1*	5.03	12.2	7.9
	2002	LG 1	E4M1_13	E4M1_13-E7M8_6	2.00	4.7	3.3
		LG 6	E1M7_2	E4M8_8-E8M7_4	2.48	5.8	3.4
		LG 7	E3M4_3	E6M8_7-E3M4_3	5.77	14.6	-5.5

[a], Confidence interval, these boundaries indicate the width of the peaks before the likelihood drops 10-fold (a LOD drop of 1.0)
[b], Percentage of variance explained by the QTL
[c], additive effect, the allele providing the higher value for the trait is coded by a positive sign for BJ-70 and a negative one for BJ-99

Analysis of segregation of an *IPMS-Bj2* locus, mapped on LG12, corresponding to the 630-bp fragment of the specific PCR amplification, on individual genotype segregation for seed gluconapin content, permits to distinguish individuals with or without gluconapin.

As seen in Fig.2, the individuals with very low or without gluconapin seed content produced a band at 630 bp, that was absent in the individuals with gluconapin.

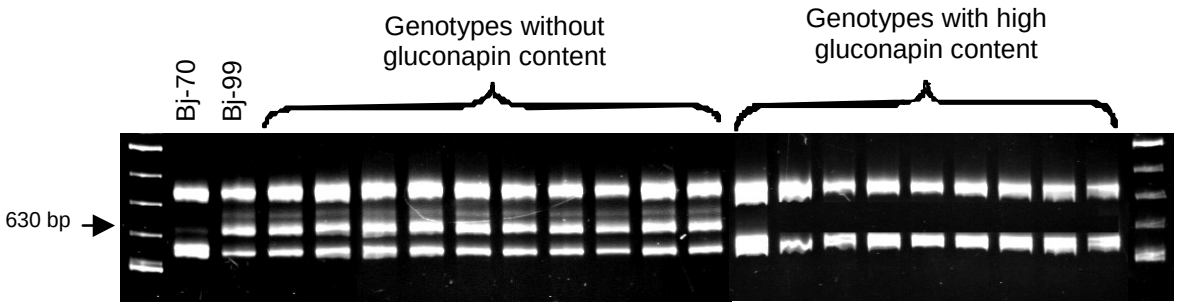

Fig. 2. Segregation of an *IPMS-Bj2* locus, corresponding to the 630-bp fragment of the specific PCR amplification on individual genotypes of *B. juncea*, using the specific PCR primers pair from *IPMS-At2*. Bj-70 and Bj-99 are the two parents of the F1 derived DH population.

DISCUSSION

The QTL detection revealed two important regions on LG12, nearly located with an *IPMS* locus, and on LG14 which control the presence or absence of gluconapin in the seed. Three QTLs were mapped for seed glucosinolate content, and particularly one on LG15 co-localised with another *IPMS* locus. The allelic effects showed that high levels of sinigrin were associated with the alleles from BJ-99 at the QTL on LG12 and LG14 and with the alleles from BJ-70 at the QTL on LG6. Only alleles from BJ-70 influenced the amount of gluconapin at the three QTLs detected. These results tend to confirm the presence of two loci for the control of sinigrin and gluconapin described by Love et al. (1990) and by Cheung et al. (1998) who mapped two common QTLs for propenyl and butenyl glucosinolates in *B. juncea*. They also suggest that two duplicated regions, analogous to G*sl-elong* genes, controlled the side-chain elongation of glucosinolates in the DH population probably due to the presence of the A and B genome. Loci on LG12 and LG14 may control the side-chain elongation step from propyl to butenyl, and loci on LG6 and LG15 may control the side-chain elongation step upstream propyl glucosinolate.

The co-segregations of candidate genes for *IMPS* genes confirmed the localisation of the QTLs and validate the candidate *IPMS* genes approach. This work provided markers of an *IPMS* gene itself and could be used for marker assisted selection to eliminate plants with gluconapin.

REFERENCES

Brunel D., N. Froger, G. Pelletier, 1999: Development of amplified consensus genetic markers (ACGM) in *Brassica napus* from *Arabidopsis thaliana* sequences of known biological function. Genome 42:387–402.

Campos, H., R. Magrath, D. McCallum, J. Kroymann, D. Scnabelrauch, T. Mitchell-Olds, and R. Mithen, 2000: α-Keto acid elongation and glucosinolate biosynthesis in *Arabidopsis thaliana*. Theor. Appl. Genet. 101, 429-437.

Cheung, W.Y., B.S. Landry, P. Raney, and G.F.W. Rakow, 1998: Molecular mapping of seed quality traits in *Brassica juncea* L. Czern. and Coss. Proc. Int. Symp. on *Brassicas*. Eds Thomas Gregoire, Acta Hort. 459, 139-147.

Lionneton, E., W. Beuret, C. Delaitre, S. Ochatt, and M. Rancillac, 2001: Efficient microspore culture and double haploid plant regeneration for the brown condiment mustard (*Brassica juncea*). Plant Cell Rep. 20, 126-130.

Lionneton, E., S. Ravera, L. Sanchez, G. Aubert, R. Delourme, and S. Ochatt, 2002: Development of an AFLP-based linkage map and localisation of QTLs for seed fatty acid content in condiment brown mustard (*Brassica juncea*). Genome 45, 1203-1215.

Love, H.K., G. Rakow, J.P. Raney, and R.K. Downey, 1990: Genetic control of 2-propenyl and 3-butenyl glucosinolate synthesis in mustard. Can. J. Plant Sci. 70, 425-429.

Magrath, R., F. Bano, M. Morgner, I. Parkin, A. Sharpe, C. Lister, C. Dean, J. Turner, D. Lydiate, and R. Mithen, 1994: Genetics of aliphatic glucosinolates. I. Side chain elongation in *Brassica napus* and *Arabidopsis thaliana*. Heredity 72, 290-299.