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Biosynthesis and heredity of rapeseed glucosinolates studied by HPCE of intact glucosinolates

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ABSTRACT

The heredity of intact individual glucosinolates in oilseed rape (Brassica napus L.) have been the subject for comprehensive studies. The glucosinolates were determined by use of a new technique based on micellar electrokinetic capillary chromatography (MECC). The parents used in the crosses were an artificial synthesized B. napus and a double low B. napus spring rape cultivar, Jaguar. Great variations have been found in F2 both with respect to the total content and the relative amounts of individual glucosinolates although progoitrin and gluconapin were the most dominant ones. In backcrossing, a sample has been found which had a level of glucosinolates close to the detection limit. Based on data obtained, we note that several genes are involved in the B. napus glucosinolate biosynthesis and backcrossing with double low material as maternal parent appeared to be the most efficient way in quality rape breeding for low glucosinolate content. Consideration of the biosynthetic steps involved in the formation of the various types of Brassica glucosinolates from the amino acid precursors- methionine, phenylalanine and tryptophan also indicates that several enzymes are involved- may be in a multienzyme complex.

INTRODUCTION

Progress, in connection with production and utilization of oilseed rape (Brassica napus L. or B. campestris L.) and products thereof, has been closely related to successful plant breeding resulting in high yielding double low rape varieties. However, plant breeding directed at oilseed rape with lower and/or changed glucosinolate contents in the seeds are still needed. Knowledge of the glucosinolate biosynthesis and heredity of glucosinolates in B. napus is essential to obtain this goal (1,2).

Parts of the glucosinolate biosynthesis are well-known, whereas other parts still need attention (1,2). Three amino acids are known as precursors for B. napus glucosinolates; methionine is precursor for the aliphatic glucosinolates (progoitrin, napoleiferin, sinigrin, gluconapin, glucobrassicanapin, methylthio-, methylsulfonyl- and methylsulfinylglucosinolates and some others), phenylalanine is precursor for gluconasturtin, and tryptophan is precursor for indol-3-ylmethylglucosinolates. In total, about 30 glucosinolates are known as B. napus constituents (3,4). This means that the glucosinolate biosynthesis involves a lot of steps for transformation of the amino acids into the final glucosinolate structures (1). Therefore, several enzymes and genes need to be involved, which gives a relatively complex heredity of glucosinolates. This work describes studies of the inheritance of individual Brassica glucosinolates after reciprocal crossing and back-crossing with use of the recently developed simple and advanced HPCE- MECC technique for determination of individual intact glucosinolates. The parents used in the crosses were an artificial synthesized B. napus and a double low spring rape cultivar, Jaguar. Great variations have been found in F2, both with respect to the total content and the relative amounts of individual glucosinolates.

MATERIAL AND METHODS

PLANTS

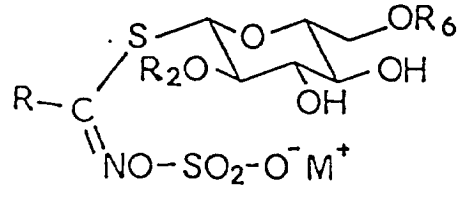
The parents used in the crosses were : an artificial synthesized Brassica napus and a double low B. napus spring rape cultivar, JAGUAR. The artificial B. napus was synthe sized by crossing Brassica alboglabra (CC genome donor) and Brassica campestris cv. Yellow sarson (AA genome donor) (5). Reciprocal crosses were made between Jaguar and the resynthesized B. napus. F1 plants were selfed and backcrossed to both parents. Parental, F1 and F2 and backcross populations were selfed by bagging. Seeds were harvested at full maturity.

Ma (Maribo) no	Description of the crosses	Number of plants
Ma 1-8	Parent; Resynt. B. napus (11-0001.0001-6019)	8
Ma 9-18	Parent; 1-9010 (Jaguar)	10
Ma 19-24	F1: 1-9010 x Resynt.B. napus (11-0007.0000.6019)	6
Ma 25-32	RF1: Resynt.B. napus x 1-9010 (11-0015.0000-6010)	8
Ma 33-91	F2: 1.9010 x Resynt.B.napus (11-0007.0002-1210)	59
Ma 92-158	- " -	67
Ma 159-215	F2: 1-9010 x Resynt.B. napus (11-0007.0003-1210)	57
Ma 216-262	- " -	47
Ma 263-296	BC: F1 x 1-9010 (11-0043.0002-6010)	34
Ma 297-341	BC: F1 x 1-9010 (11-0043.0003-6010)	45
Ma 342-401	BC: 1-9010 x F1 (11-0044.0002-6010)	60
Ma 402-462	BC: 1-9010 x F1 (11-0044.0003-6010)	61
Ma 463-522	BC: F1 x Resynt.B. napus (11-0045.0002-6010)	60
Ma 523-584	BC: F1 x Resynt.B. napus (11-0045.0003-6010)	62

ANALYTICAL METHODS

Micellar electrokinetic capillary chromatography (MECC) was used for analysis of individual glucosinolates after a simple sample purification process (6). The content of individual glucosinolates in seeds was calculated by a procedure based on the use of two internal standards and response factors.

Fig. 1. Structure of glucosinolates.
R = side chain with structural resemblance to the parent amino acids ;
R2 and R6 = H or acyl derivatives ;
M+ = cation. (Reproduced from Ref. 4)



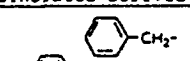
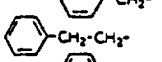
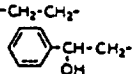
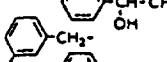
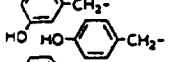
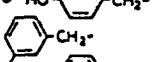
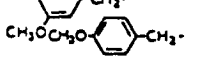
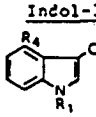
RESULTS AND DISCUSSION

In double- as in single low rapeseed, gluconapin, glucobrassicanapin, progoitrin and napoleiferin are the glucosinolates traditionally considered, but in addition to these, methylsulphinyl- and indol-3-ylmethylglucosinolates, especially 4-hydroxyglucobrassicin, can be quantitatively dominating (3,4). These glucosinolates have, owing to analytical reasons (4), often been excluded from plant breeding programmes and studies of glucosinolate heredity (1,2). With new analytical techniques, based on high performance capillary electrophoresis (HPCE), which is now available as micellar electrokinetic capillary chromatography (MECC) for glucosinolate analysis (6), it is possible with reasonable resources to go into more detailed studies of the glucosinolate biosynthesis and heredity.

In Brassica, about 30 different glucosinolates occur with structures, names and numbers, as presented elsewhere (3,4). Among which No. 17, 23, 24, 26, 27 and methionine derived glucosinolates are the quantitatively dominant ones.

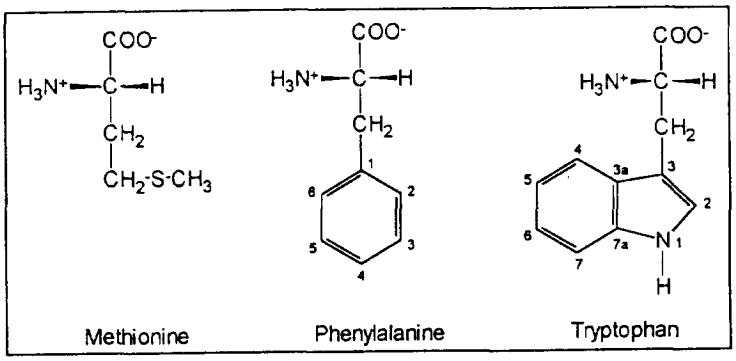
For the phenylalanine derived phenethylglucosinolate, 17, and all of the methionine derived Brassica glucosinolates, the chain elongation steps are included in the glucosinolate biosynthesis. The chain elongated amino acids have, however, not been established as free

Table 1. Selected glucosinolates important for the quality of rapeseed and other cruciferous crops. (Reproduced from Ref. 4)

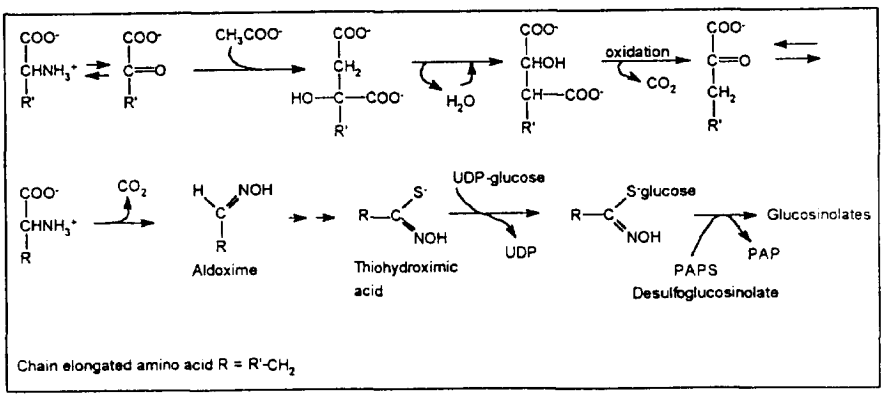
No.	Structure of R group		Glucosinolates: Semisystematic names	Trivial names
	Glucosinolates derived from methionine:			
1	CH_2=CH-CH_2-		Allylglucosinolate	Sinigrin
2	CH_2=CH-CH_2-CH_2-		But-3-enylglucosinolate	Gluconapin
3	CH_2=CH-CH_2-CH_2-CH_2-		Pent-4-enylglucosinolate	Glucobrassicanapin
4	CH_2=CH-CH(OH)-CH_2-		(2R)-2-Hydroxybut-3-enylglucosinolate	Progoitrin
5	-- " --		(2S)-2-Hydroxybut-3-enylglucosinolate	Epiprogoitrin
6	CH_2=CH-CH_2-CH(OH)-CH_2-		(2R)-2-Hydroxypent-4-enylglucosinolate	Napoleiferin
7	CH_3-S-CH_2-CH_2-CH_2-		3-Methylthiopropylglucosinolate	Glucoibervirin
8	CH_3-S-CH_2-CH_2-CH_2-CH_2-		4-Methylthiobutylglucosinolate	Glucoerucin
9	CH_3-S-CH_2-CH_2-CH_2-CH_2-CH_2-		5-Methylthiopentylglucosinolate	Glucoberteroin
10	CH_3-SO-CH_2-CH_2-CH_2-	Δ	3-Methylsulphinylpropylglucosinolate	Glucoiberin
11	CH_3-SO-CH_2-CH_2-CH_2-CH_2-	Δ	4-Methylsulphinylbutylglucosinolate	Glucoraphanin
12	CH_3-SO-CH_2-CH_2-CH_2-CH_2-CH_2-	Δ	5-Methylsulphinylpentylglucosinolate	Glucoalyssin
13*	CH_3-SO-CH=CH-CH_2-CH_2-	o Δ	4-Methylsulphinylbut-3-enylglucosinolate	Glucoraphenin
14	CH_3-SO_2-CH_2-CH_2-CH_2-		3-Methylsulphonylpropylglucosinolate	Glucocheirolin
15	CH_3-SO_2-CH_2-CH_2-CH_2-CH_2-		4-Methylsulphonylbutylglucosinolate	Glucoerysolin
	Glucosinolates derived from phenylalanine:			
16			Benzylglucosinolate	Glucotropaeolin
17			Phenethylglucosinolate	Gluconasturtiin
18*		□	2-Hydroxy-2-phenylethylglucosinolate	Glucobarbarin
19			m-Hydroxybenzylglucosinolate	Glucolepigramin
20			p-Hydroxybenzylglucosinolate	Sinalbin
21			m-Methoxybenzylglucosinolate	Glucolimnanthin
22			p-Methoxybenzylglucosinolate	Glucoaubrietin
	Indol-3-ylmethylglucosinolates biosynthetically derived from tryptophan:			
23		(R_1=R_4=H	Indol-3-ylmethylglucosinolate	Glucobrassicin
24		R_1=OCH_3; R_4=H	N-Methoxyindol-3-ylmethylglucosinolate	Neoglucobrassicin
25		R_1=SO_3^-; R_4=H	N-Sulphoindol-3-ylmethylglucosinolate	Sulphoglucobrassicin
26		R_1=H; R_4=OH	4-Hydroxyindol-3-ylmethylglucosinolate	4-Hydroxyglucobrassicin
27		R_1=H; R_4=OCH_3	4-Methoxyindol-3-ylmethylglucosinolate	4-Methoxyglucobrassicin

*Occur also as cinnamoylderivatives
Δ(R)-configuration at the sulphinyl group
o trans-E-configuration
□ (2S)-configuration in glucobarbarin and (2R)-configuration in glucosibarin

amino acids accumulated in the plants. Amino acids with the various changes in the side chains, R-groups, as known for the corresponding glucosinolates (4), have neither been established as free amino acids in the plants. The oxidation of the methionine sulfur to the chiral methylsulfinyl group seems furthermore to give opposite chirality for the free amino acids and the glucosinolates (1), and the desaturation step does not necessary involve a loss of the methylthio-group (1) as shown in the following figures. The hydroxylation of aliphatic glucosinolates occurs also as a stereospecific step, although 2-hydroxy-substituted glucosinolates of both chiralities occur in Brassica as well as in other plants (4). Several steps in the biosynthesis of glucosinolates are thus well-known (1,2), but several details remain to be elucidated and it seems likely that the glucosinolate biosynthesis occurs in a multi-enzyme/protein complex.

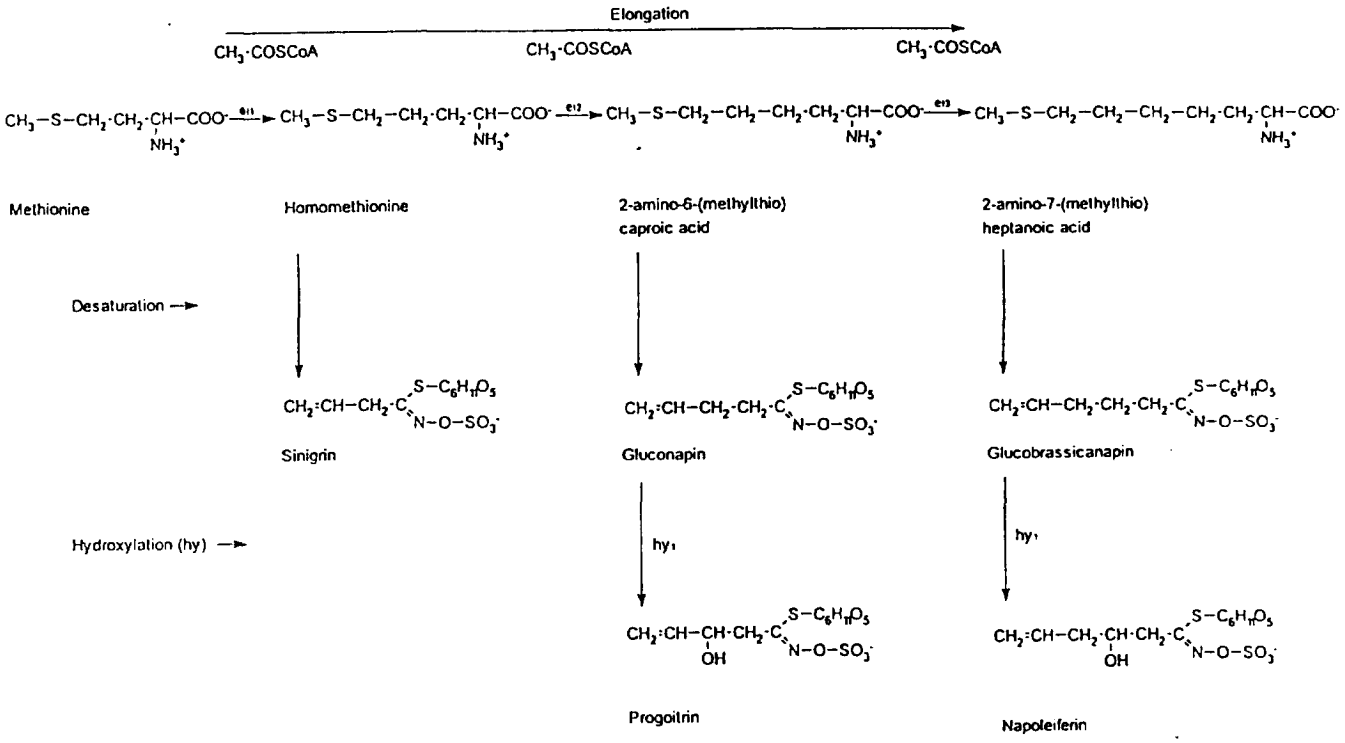


Three precursors for glucosinolate biosynthesis in Brassica



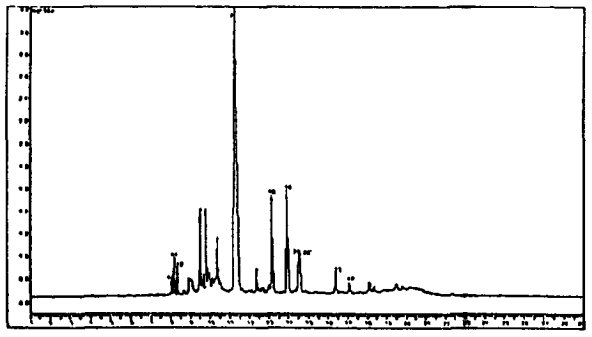
The structures of the three amino acids which are precursors for glucosinolate biosynthesis in Brassica are shown in the figure above as are the more or less well-known steps of the generally accepted steps in the glucosinolate biosynthesis discussed in more details elsewhere (1,2).

Structures and names of glucosinolates produced from methionine after 1,2 or 3 chain elongation steps followed by desaturation and 2-hydroxylation are shown in the following figure.

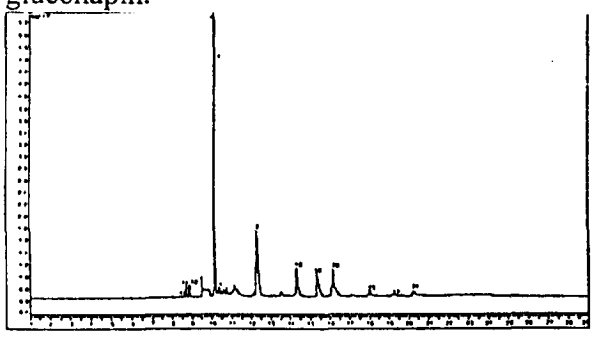


Inheritance of both total and individual glucosinolate contents needs thus attention with determination of both type and amounts of the compounds by use of reliable methods. Intact glucosinolates in seeds of the individual plants from the crossing have been determined by a fast and simple HPCE-MECC technique (6). Both total glucosinolate content and glucosinolate pattern showed great variations, and examples of the chromatograms are shown in the following figures.

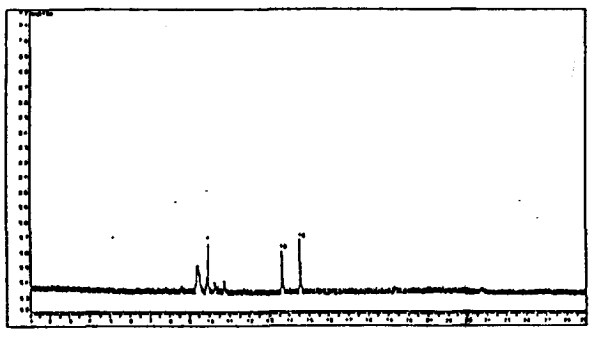
A backcrossing sample with progoitrin as the dominant one but very low content of gluconapin.



A plant in backcrossing with gluconapin as the dominant glucosinolate.



A normal sample with No.4, 2,and 26 as the dominant glucosinolate as well as the three sulphinyl glucosinolates No.10,11,and 12.

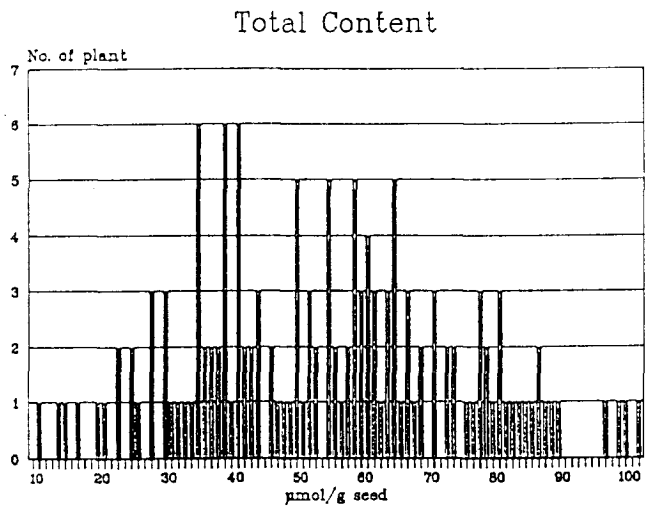


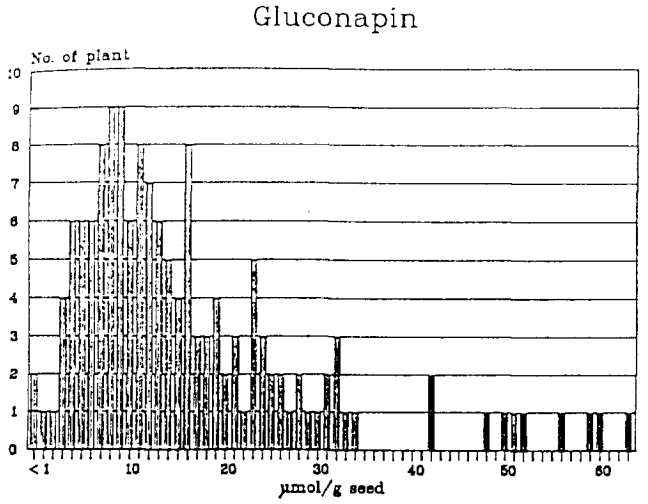
A sample from backcrossing with Jaguar as the maternal parent only containing a very low content of progoitrin.

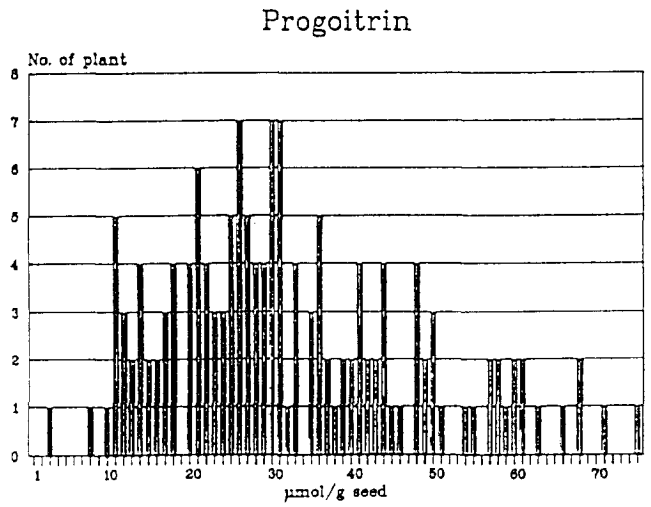
Segregation of total glucosinolate content in F2 is presented in the following figures. A data analysis showed that there were more than 20 genes controlling the inheritance of the total glucosinolate content. This result is different from that given in the literature and proposing that 2-6 genes are involved (7,8,9).

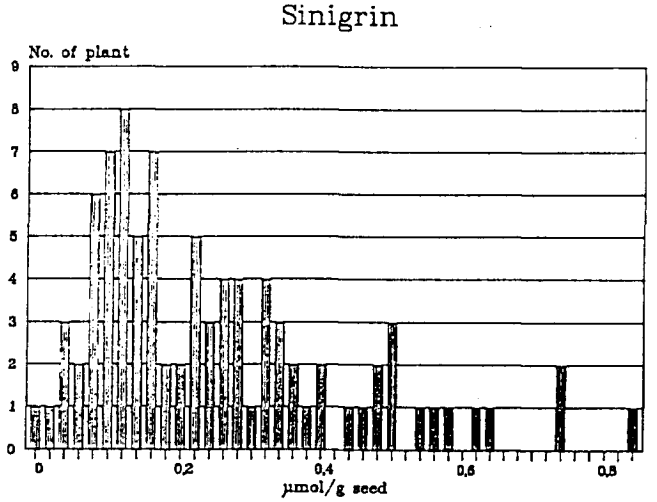
The segregation in F2 shown from progoitrin, guconapin and sinigrin suggested that at least 3 genes were involved in the heredity of each glucosinolate. Epistasis existed in glucosinolate heredity. The high content was dominant to the lower one in most glucosinolates studied with the exception of gluconapin.

A more detailed date analysis of indolyl glucosinolate heredity and effects of the cytoplasm is still being carried out. It seems, however, that the glucosinolate heredity in Brassica is much more complicated than it had previously been proposed with only 2-6 genes control (7,8,9).









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