INHERITANCE OF ALKENYL GLUCOSINOLATES IN TRADITIONAL AND MICROSPORE-DERIVED DOUBLED HAPLOID POPULATIONS OF BRASSICA JUNCEA L. CZERN AND COSS.

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ABSTRACT

Alkenyl glucosinolate segregation patterns were studied in F_2, BC_1, and F_1-derived doubled haploid populations arising from a cross between a high glucosinolate East Indian cultivar, RLM 514, and a low glucosinolate *B.juncea* breeding line. A wide array of total glucosinolate levels were observed consisting of various combinations propenyl, butenyl, and pentanyl glucosinolates. A comparison of segregation in the three genetic populations indicated that doubled haploidy can be used effectively to elucidate the complex inheritance patterns of these compounds.

INTRODUCTION

Following the development of canola quality *Brassica juncea* through interspecific hybridization (Love *et al* 1990 a), cultivar development in this species is under way in Canada .The benefits of this new crop include drought tolerance, shattering and blackleg resistance, yellow seed color and higher seed yield than conventional canolas. At the University of Alberta, the doubled haploid (DH) system is being used in addition to the traditional breeding methods, to accelerate cultivar development in *B.juncea*. We have also used microspore derived DH lines in a study to explore the genetics of several characters. The results on the inheritance of seed color and leaf hairiness in *B.juncea* have already been reported (Thiagarajah and Stringam 1993), and we report here the results from that study on the genetics of glucosinolate expression in this species.

MATERIALS AND METHODS

The East Indian *B.juncea* cultivar RLM514, which has a high seed glucosinolate content (Table 1) was crossed reciprocally with a canola quality *B.juncea* breeding line from the University of Alberta breeding program. The growth conditions used for the F_1 plants and the microspore culture procedure used to develop the DH lines have already been described (Thiagarajah and Stringam 1993). Seed for glucosinolate analysis was harvested from the parental lines, the backcross progeny, F_1 and F_2 plants,and from DH lines grown in the field. Glucosinolate composition of seed harvested from individual open pollinated plants was determined by gas liquid chromatography (Daun and Mcgregor 1983). The average of the glucosinolate contents of three randomly harvested plants from each DH line was used to represent the glucosinolate content of that line.

RESULTS AND DISCUSSION

Among the 376 F_2 plants harvested, only one yielded seed having a low glusosinolate (14.5 µmol/g total alkenyl) content. This suggests that at least 4 genes in the recessive

condition are required for the expression of low alkenyl glucosinolate levels, and it is likely that the number of F_2 plants sampled may not have been adequate to analyze the inheritance of a multigenic trait of this nature.

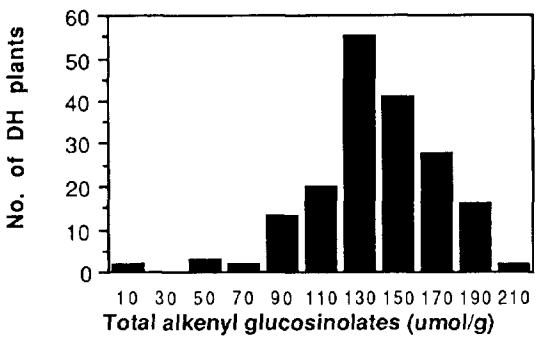
Seed harvested from F_1 plants of both reciprocal crosses contained similar levels of total alkenyl glucosinolates (Table 1), indicating that glucosinolate synthesis was under the control of nuclear rather than cytoplasmic genes. No clear differences in the glucosinolate composition were apparent in the seed derived from the reciprocal crosses.

TABLE 1. Alkenyl glucosinolate composition of seed from parental and F_1 plants (mean±std. error µmol/g)

	No. of Plants	Total Alkenyl	2-Propenyl	3-Butenyl	4-Pentenyl	2Hydroxy butenyl
P1*	64	174.4±1.8	48.7±2.4	123.4±3.0	1.3±0.04	0.7±0.02
P2	44	4.6±0.3	1.0±0.2	2.5±0.2	0.9±0.04	0.1±0.03
P1 x P2	51	163.4±2.4	71.0±3.7	88.0±4.5	3.2±0.5	0.9±0.04
P2 x P1	13	161.9±3.4	87.7±6.9	70.0±5.9	3.3±1.0	0.8±0.07

*P1= RLM514 ; P2= Low glucosinolate parent

Fig.1. Frequency distribution for total alkenyl glucosinolates in DH lines



The glucosinolate content of the DH lines was skewed towards higher total alkenyl glucosinolate levels (Fig.1). In some DH lines, individual glucosinolate levels for propenyl or pentenyl, surpassed the levels found in the high parent. The population mean for total alkenyl glucosinolates for the DH lines in this study was 79.7% of the high parent value. Siebel and Pauls (1989) who observed a similar trend among DH lines derived from low x high glucosinolate crosses in *B.napus*, suggested that the large proportion of high glucosinolate lines may be due to high glucosinolate levels displaying partial dominance and/or overdominance to low glucosinolate levels.

Segregation ratios observed in the DH lines for total alkenyl glucosinolates (Table 2) fitted both the 1:31 and 1:255 ratios, indicating that 5 to 8 recessive alleles control the complete absence of alkenyl glucosinolates in *B.juncea*. The segregation observed in the backcross progeny suggests the involvement of 6 to 9 recessive alleles. Multiple gene

action in glucosinolate expression in *B.juncea* has also been reported by Love *et al.*(1990b).

TABLE 2. Segregation ratios and chi-square tests for alkenyl glucosinolate levels in DH and backcross populations.

	P2 range µmol/g		Observed ratio (-):(+)*	Expected ratio	χ^2	P
Total alkenyl	2.5-14	DH	2:180	1:31	2.457	0.05-0.2
				1:255	2.353	0.05-0.2
		BC	2:214	1:63	0.572	0.2-0.5
				1:511	5.912	0.01-0.05
2-Propenyl	0.1-5.6	DH	27:155	1:7	0.907	0.2-0.5
		BC	29:187	1:7	0.169	0.5-0.8
3-Butenyl	1.4-8.6	DH	65:117	1:3	11.14	<0.01
		BC	40:176	1:3	4.84	0.01-0.05

*(-)= within P_2 range; (+)= outside P_2 range

A theoretical ratio of 1:7 expected with 3 recessive genes, fitted the 2-propenyl glucosinolate segregation pattern in both the DH and the BC populations, suggesting the involvement of three recessive alleles in controlling low propenyl levels. Although a theoretical ratio of 1:3 did not fit the observed ratio for the 3-butenyl glucosinolate classes in the DH lines, there was an acceptable fit in the case of the BC progeny, suggesting the action of two recessive genes.

The data from this study indicate the complexity of glucosinolate inheritance in *B.juncea*. Although the high glucosinolate parent used in the study had high levels of both 2-propenyl as well as 3-butenyl glucosinolates, a few of the F_1-derived DH lines which had high glucosinolate levels were either predominantly propenyl or butenyl types, suggesting complex developmental pathways. These DH lines could be useful in further studies on the inheritance of glucosinolates in *B.juncea*. This study shows that doubled haploidy could be usefully employed in *B.juncea* breeding programs not only to efficiently recover deisirable genotypes determined by multiple recessive genes, but also in the genetic analysis of complex traits.

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