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STUDIES ON GLUCOSINOLATES IN CLUBROOT RESISTANT SELECTIONS AND SUSCEPTIBLE COMMERCIAL CULTIVARS OF CABBAGES*

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SUMMARY

Heads of 59 commercial cabbage cultivars, all susceptible to clubroot disease, and of 86 individual clubroot resistant cabbage plants of various breeding selections were analysed for their composition in glucosinolates, determined by quantifying their hydrolytic breakdown products, thiocyanate, goitrin, and volatile isothiocyanates. The mean thiocynate ion content was significantly lower in the breeding selections (120 μg/g dry weight) than in the commercial cultivars (204 μg/g). In contrast, the mean goitrin content was significantly higher in the breeding selections (193 μg/g) than in the commercial cultivars (35 μg/g). Similar to goitrin, the range of volatile isothiocyanates and total glucosinolate were higher in the breeding selections, but the mean contents of each were not statistically different between selections and cultivars. Fourteen cultivars and four selections were found to be free of goitrin; three cultivars, but no breeding selection were free of volatile isothiocyanates. The breeding selections will provide germplasm for breeding new clubroot resistant and low glucosinolate cultivars.

INTRODUCTION

Clubroot, caused by *Plasmodiophora brassicae* WOR., is one of the most destructive disease of cruciferous crops in Europe and North America. In Canada, the most prevalent races are 2 and 6, coded as populations ECD 16/02/31 and 16/02/30, respectively (BUCZACKI et al., 1975). Presently, all commercial cultivars of cabbages are susceptible to ECD 16/02/31. CHIANG et al. (1977) transferred through interspecific hybridization a dominant gene responsible for resistance to *P. brassicae* race 2 from rutabaga (*Brassica napus* L., 2n = 38) to cabbage (*B. oleracea* L. ssp. *capitata* L., 2n = 18). Cabbage selections resistant to both ECD 16/02/31 and 16/02/30 have already been developed (CHIANG & CRETE, 1983).

Cruciferous plants typically contain glucosinolates, a class of secondary plant meta-

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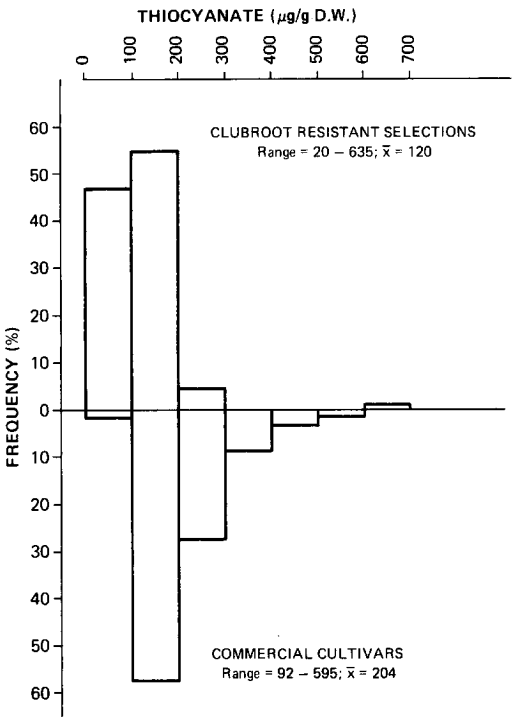

Fig. 1. Variation of thiocyanate ion (percent frequency in interval concentrations of 100 μg/g dry weight) in cultivars and clubroot resistant selections of cabbages.

bolites which upon cell rupture or disorganization are hydrolysed by myrosinase yielding a number of major breakdown products, including isothiocyanates (mustard oils), thiocyanates and oxazolidinethiones (ETTLINGER & KJAER, 1968; UNDERHILL, 1980). FENWICK et al. (1982) have reviewed the contribution of glucosinolate breakdown products to the characteristic flavor and taste and to the biological effects of these crops. Different glucosinolates are known to have different effects, including toxicity to herbivorous insects feeding on crucifers, or different intensity of taste (RODMAN, 1980). Studies have associated the development of goiter with consumption of cruciferous crops (WRIGHT & SINCLAIR, 1958; STANBURY, 1969; PAXMAN & HILL, 1974; ANONYMOUS, 1975).

Evidence indicates that glucosinolates are genetically controlled, and that breeding to reduce their amounts should improve the quality of cruciferous crops (JOHNSTON & JONES, 1966; KEHR, 1973). In fact, varieties of low glucosinolate rapeseed (*B. campestris* and *B. napus*) have been bred (ROBBELEN & THIES, 1980). However, there is a lack of knowledge on the inheritance of glucosinolates in cruciferous vegetable crops.

We are now studying the composition of glucosinolates and the nature of their inheritance in cabbage and related cruciferous vegetables crops to improve the quality of clubroot resistant lines by selecting those that are also low in glucosinolates. In this context, this study compared the glucosinolate composition of 59 commerical cabbage cultivars and 86 individual clubroot resistant cabbage plants sampled from 29 breeding selections. The glucosinolates were quantified in terms of their breakdown products, thiocyanate ion, goitrin and volatile isothiocyanates.

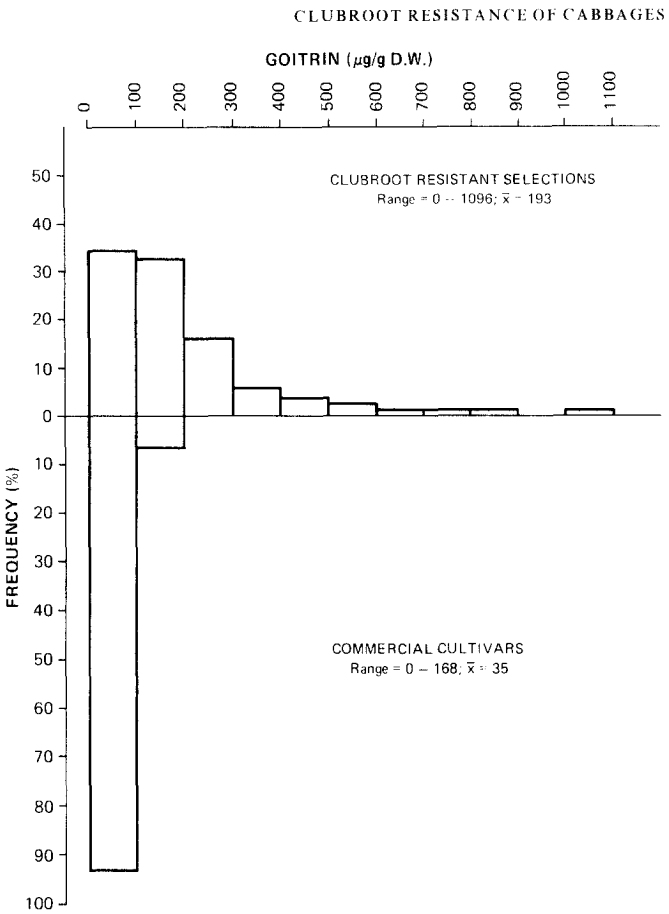

Fig. 2. Variation of goitrin (percent frequency in interval concentrations of 100 μg/g dry weight) in cultivars and clubroot resistant selections of cabbages.

MATERIALS AND METHODS

Seeds of commerical cabbage cultivars were purchased from or donated by seed companies; those of breeding selections were harvested from the selfed 4th backcross (B_4) progenies in our breeding stocks (*Brassica napus* × *B. oleracea* ssp. *capitata* → F_1♀ (male sterile) × 2x-cabbage → B_1♀ (ms) × 2x-cabbage → B_2♀ (ms) × 2x-cabbage → B_3♂ × 2x-cabbage → B_4 selfing; for detailed pedigree see CHIANG & CRETE, 1983). Seeds were sown on May 10, 1982, in cold frames at the St-Jean Research Station. On June 6, seedlings of the breeding selections were transplanted to replicated clubroot pathogen-infested field plots; seedlings of the commercial cultivars were transplanted to non-infested plots. Cutural practices were followed according to Quebec provincial recommendations (C.P.V.Q. 1982, Agdex 250/20, p. 38–46; C.P.V.Q. 1983–84, Agdex 250–605, p. 27–40).

Three plants of each cultivar from two replicated plots were harvested as they became mature, July 29 to October 6; 86 individual clubroot-resistant plants of the var-

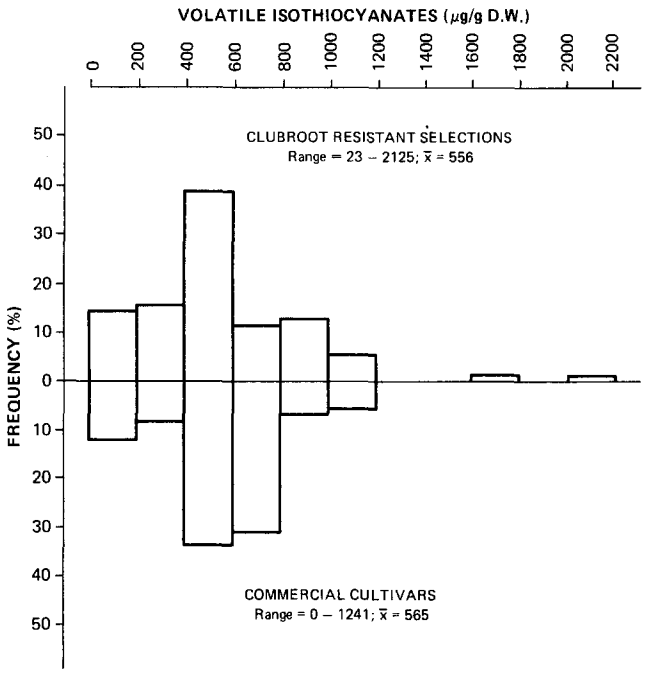

Fig. 3. Variation of volatile isothiocyanates (percent frequency in interval concentrations of 200 μg/g dry weight) in cultivars and clubroot resistant selections of cabbages.

ious breeding selections, sampled for their desirable horticultural charactersitics, were harvested between September 22 and October 20. At Macdonald College, three respresentative tissue subsamples (ca. 70 g of wedge-shaped portions of head tissue) were combined from the three plants of each cultivar per replicated plot, or taken from the head of each clubroot-resistant plant. The first sub-sample was extracted immediately by homogenizing in a Waring blender with distilled water (1:3, wt/vol.). The extract was then clarified, and determined in duplicate for thiocyanate ion content, expressed as μg per gram of oven-dry weight of tissue; dry weight was determined from the second subsample (CHONG & BIBLE, 1974). The third subsample was cut into small pieces, frozen by immersion in liquid nitrogen, and stored in a freezer at −20 °C. The frozen subsamples was later freeze-dried, ground into powder, and stored in the freezer until analysed for goitrin and isothiocyanates (including those which cyclize to form goitrin) after glucosinolate hydrolysis with myrosinase (Ju et al., 1980). The goitrin content in terms of μg equivalents of progoitrin was substracted from isothiocyanate total in terms of μg equivalents of 3-butenyl-isothiocynate to obtain an estimate of volatile isothiocyanates (MCGREGOR, 1978). Results for goitrin and volatile isothiocyanates are expressed as μg/g 2-hydroxy-3-butenyl isothiocyanate and 3-butenyl-isothiocyanate, respectively, on freeze-dry weight basis.

RESULTS

Comparative data for variation of the glucosinolate products and for total glucosino-

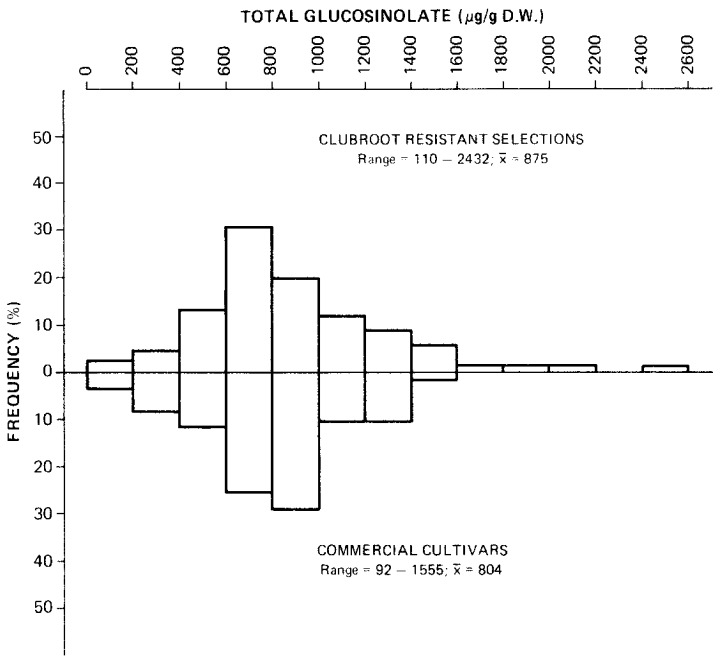

Fig. 4. Variation of total glucosinolate (percent frequency in interval concentrations of 200 μg/g dry weight) in cultivars and clubroot resistant selections of cabbages.

late in cabbage cultivars and breeding selections are presented in terms of percent frequency in interval concentrations of 100 or 200 μg/g dry weight (Fig. 1–4).

The mean thiocynate ion content (Fig. 1) was significantly lower in the breeding selections (120 μg/g) than in the commercial cultivars (204 μg/g) as indicated by *t*-test ($t' = 6.56$; $P \leqslant 0.01$), taking into consideration the variance of the two sample populations (STEEL & TORREY, 1960). While 47% of the breeding selections had thiocyanate ion content in the lowest (0–99 μg/g) interval range (Fig. 1), only 1.7% or one cultivar was in this concentration range; 58% of the commerical cultivars were in the range of 100–199 μg/g. In contrast, the mean goitrin content (Fig. 2) was significantly higher in the breeding selections (193 μg/g) than in the commerical cultivars (35 μg/g) ($t' = 7.58$; $P \leqslant 0.01$). Most (94%) of the commercial cultivars had goitrin content in the lowest (0–99 μg/g) interval range (Fig. 2) with 14 found to be goitrin-free; 34% of the breeding selections were within this interval range with only four free of goitrin. Similar to goitrin, the range of volatile isothiocyanates (Fig. 3) and total glucosinolate (Fig. 4) were higher in the breeding selections, but the mean contents of each were not statistically different between selections and cultivars. Three cultivars, but no breeding selection, were found to be free of volatile isothiocyanates.

Tables 1 and 2 list 18 commercial cultivars and 19 breeding selections, respectively, with the contents of one or more glucosinolate entries $\leqslant$ mean content of the sample of population of each entry minus 1 standard deviation. Cultivars and selections thus categorized were all goitrin-free. Three cultivars, Earlibird, Tastie, and Hybrid 1561, were lacking both goitrin and volatile isothiocyanates. Earlibird and Tastie (Table

Table 1. Commercial cabbage cultivars low in or lacking one or more glucosinolate products.

Cultivar	Type	µg/g dry weight			
		thiocyanate ion	goitrin	volatile isothio-cyanates	total gluco-sinolate
Autoro	F_1	325	0*	725	1050
Charlestone Wakefield	Open pollinated	127	0*	217*	344*
Danish Ball Head	Open pollinated	231	0*	389	620
Earlibird	F_1	104*	0*	0*	104*
Excel	F_1	188	14	126*	328*
Gourmet	F_1	193	0*	58*	251*
Hybrid 1561	F_1	377	0*	0*	377*
Hyjula	F_1	112	0*	560	672
Ice Queen	F_1	102*	27	1207	1336
Jumbo	Open pollinated	109	0*	554	663
Mascotte	F_1	350	5	176*	531
Mercury	F_1	229	0*	908	1137
Prime Pack	F_1	121	0*	143*	264*
Resistant Danish	F_1	150	0*	366	516
Superette	F_1	106*	9	460	575
Tastie	F_1	92*	0*	0*	92*
Titanic	F_1	132	0*	612	744
U-neek Savoy	Open pollinated	474	0*	491	965

*Cultivars containing glucosinolate product $\leqslant$ mean of sample population – 1 standard deviation, i.e. $\leqslant$ 107 µg/g thiocyanate ion; 0 µg/g goitrin; $\leqslant$ 287 µg/g volatile isothiocyanates; $\leqslant$ 495 µg/g total glucosinolate.

1), and plant No. B-21 of selection No. 137 (Table 2) had total glucosinolate in the 0–199 µg/g interval range.

It is worth while noting that results of seed analysis of the commercial cultivars indicated substantially higher contents of thiocyanate ion (range 175–513, $\bar{x}$ = 337), goitrin (range 36–1662, $\bar{x}$ = 485), volatile isothiocyanates (range 1300–5261, $\bar{x}$ = 2943), and total glucosinolate (range 1439–6130, $\bar{x}$ = 3765) (unpublished data) than in the heads of the cultivars (Fig. 1–4). In a study of 50 cabbage cultivars, TOOKEY et al. (1980) reported significant correlations (absolute value of r ranging from 0.30 to 0.73) between individual glucosinolates in head and seed, including precursors of goitrin and thiocyanate ion, and also total glucosinolate. In the present study, a low but statistically significant correlation (r = 0.33; $p < 0.05$; *df* 57) was found only between head and seed goitrin.

DISCUSSION

Studies by BUTCHER et al. (1974, 1976) suggested that the susceptibility to clubroot in various cruciferous species was related to the presence of glucosinolates, particularly the indolyl glucosinolates which hydrolyse to yield thiocyanate ion. The abnormal growth symptoms of clubroot tissues are associated with higher than normal levels

Table 2. Clubroot resistant cabbage selections low in or lacking one or more glucosinolate products.

Selection no.	Plant no.	µg/g dry weight			
		thiocyanate ion	goitrin	volatile isothiocyanates	total glucosinolate
103	B-1	47*	257	224	528
104	B-3	20*	442	129*	591
	B-28	39*	18	348	405*
107	B-5	113	743	194*	1050
108	B-6	120	424	125*	669
	B-7	247	133	96*	476
	B-32	77	0*	91*	168*
112	B-36	53	90	253	396*
113	B-88	68	35	331	434*
114	B-38	136	168	72*	376*
115	B-40	165	371	142*	678
118	B-11	153	62	87*	302*
	B-13	44*	424	718	1186
124	B-45	34*	513	1602	2149
	B-46	33*	274	2125	2432
129	B-17	86	115	216	417*
	B-18	89	0*	114*	203*
135	B-20	635	0*	23*	658
137	B-21	75	0*	35*	110*

*Selections containing glucosinolate product $\leq$ mean of sample population – 1 standard deviation, i.e. $\leq$ 49 µg/g thiocyanate ion; $\leq$ 5 µg/g goitrin; $\leq$ 211 µg/g volatile isothiocyanates $\leq$ 471 µg/g total glucosinolate.

of auxins, released largely from these glucosinolates (BUTCHER et al., 1974). Observations of CHONG et al. (1981) suggest that cabbage plants with a high degree of clubroot resistance and derived from a similar genetic background as those used in the present study were also low in thiocyanate ion. The result of Fig. 1 further confirms this finding. The generally higher amounts of goitrin in the breeding selections may indicate also a possible association of goitrin content with clubroot resistance.

Goitrin, one of the most potent of natural goitrogens, derived from 2-hydroxy-3-butenyl-glucosinolate (progoitrin), is a predominant glucosinolate in seed of rapeseed (MCGREGOR, 1978) and in edible root of rutabaga (MULLIN et al., 1980) but is present in only relatively small quantities in head of commercial cabbage cultivars (Fig. 2; VANETTEN et al., 1976, 1980). This suggests that the general occurrence of higher contents of goitrin, and perhaps also of volatile isothiocyanates, in the breeding selections occurred through inheritance from the original cross involving rutabaga (CHIANG et al., 1977). The lower amounts of thiocyanate ion and higher amounts of goitrin in the breeding selections, and the reverse situation in the commercial cultivars, may indicate that the precursor glucosinolates of these products are controlled by different genes since the genetic background of the two populations are different. Glucosinolate composition in seed of rapeseed has been found to be under polygenic control, with complex segregation pattern in the F_2's (KONDRA & STEFANSSEN, 1970; JOSEFSSON,

1973). According to RODMAN et al. (1981) variation in certain glucosinolates of *Strephanthus* sp. may be a reflection of relatively minor enzymatic and also of genetic differences.

This study demonstrated important differences in composition and content of glucosinolates in clubroot resistant breeding selections and susceptible cultivars of cabbage. Furthemore, this study also identified 18 commerical cabbage cultivars (Table 1) and 19 plants of breeding selections (Table 2) that were low or lacking one or more glucosinolate products, or those low in total glucosinolate content. Similar to Hybrid Petite White turnips which was found to be lacking goitrin (CHONG et al., 1982), these genotypes will serve as germplasm in the development of low glucosinolate cabbages and related cruciferous vegetable crops. In particular, the breeding selections will provide the germplasm for breeding new clubroot resistant and low glucosinolate cultivars. This is important because of increasing concern for the quality of food, including contents of both beneficicial and harmful natural constituents in new cultivars (KEHR, 1973; HANSEN, 1974).

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