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GLUCOSINOLATES OF WILD AND CULTIVATED *BRASSICA* SPECIES

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Key Word Index—*Brassica oleracea*; *B. rapa*; Brassicaceae; chemotaxonomy; glucosinolates; plant domestication.

Abstract—Major differences were found in the occurrence of glucosinolates in the leaves of different wild species of the *Brassica oleracea* ($2n = 18$) group. The distribution of glucosinolates supports the present classification with the exception of the inclusion of both Tunisian and Sardinian populations within *B. insularis*. The results also support the hypothesis of a multiphyletic origin of cultivated forms of *B. oleracea* from a number of wild species. In contrast, wild populations of *B. rapa* had very similar glucosinolate contents. Wild populations had a considerably higher total glucosinolate content than cultivars which may limit their usefulness in breeding programmes.

INTRODUCTION

The wild Mediterranean *Brassica* species with chromosome number $2n = 18$ form a polymorphic aggregate of species belonging to the *B. oleracea* group. Some may contain agronomically important genes for disease resistance and other characteristics [1, 2], and their conservation has been given high priority [2, 3]. Hybrids between wild and cultivated forms of *B. oleracea* are at least partially fertile [4, 5]. *B. napus*-like plants ($2n = 38$), derived from *in vitro* culture of embryos from crosses between *B. rapa* ($2n = 20$) and *B. cretica* ($2n = 18$), a wild species of the *B. oleracea* group, have also been produced [6] and may be valuable in broadening the narrow genetic base of oilseed rape, *B. napus* subsp. *oleifera* [2].

Previous studies have indicated that wild brassicas may contain high levels of glucosinolates [7, 8], which may limit their usefulness in breeding programmes. Glucosinolates are an important group of sulphur containing glycosides (Fig. 1) which are distributed throughout the Brassicaceae. Following tissue damage, they are hydrolysed by the endogenous enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) to a variety of products [9], some undesirable. For example, the isothiocyanates derived from the hydrolysis of alkenyl glucosinolates such as sinigrin and gluconapin can reduce palatability of products such as rapemeal. Another problem is the goitrogenicity of the thiocyanate ion, derived from indole glucosinolate hydrolysis, and vinyloxazolidinethione, derived from the hydrolysis of the hydroxyalkenyl glucosinolate, progoitrin. These problems have led to attempts to reduce or eliminate glucosinolates in crops such as oilseed rape. Recent studies, however, have indicated that many of these hydrolysis products are associated with resistance to fungal pathogens [5].

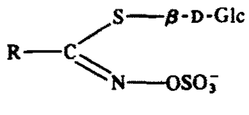

Fig. 1. General structure of glucosinolates (variations of the side chain, R, are presented in Table 1).

The wild *Brassica* species of the *B. oleracea* group are usually divided into 11 species [10] but the phylogenetic relationships between the wild taxa is uncertain. Cultivated forms of *B. oleracea* were previously believed to have had a monophyletic origin from wild western European *B. oleracea*. However, on the basis of morphological studies, it has been suggested that cultivated varieties have a polyphyletic origin from a number of wild species [4, 8]. Since previous studies on the occurrence of glucosinolate hydrolysis products have proved useful in chemotaxonomic studies of *Brassica* [8, 11, 12] and other genera [13, 14], an investigation into the patterns of glucosinolates in wild populations might indicate the phylogenetic relationships between the wild species and the origin of cultivated forms, in addition to identifying the best sources of genes to use in breeding programmes.

This study reports on the levels and distribution of glucosinolates in different populations of the *B. oleracea* group, in a smaller number of wild populations of *B. rapa*, and in cultivated forms of these two species.

RESULTS AND DISCUSSION

Nine species of glucosinolates were found in the sampled plants (Table 1). With the exception of *B. oleracea* cv. January King, wild brassicas of both the *B. oleracea* group and *B. rapa* had higher total glucosinolate content than cultivated forms (Tables 2 and 3). The high levels of glucosinolates within wild brassicas may deter plant breeders from using them as a source of other useful genes. However, little is known about the genetics of glucosinolate accumulation and it may be possible to reduce undesirable glucosinolates to acceptable limits by crossing with low glucosinolate cultivars.

The low levels of glucosinolates within some of the cultivated *B. rapa* and *B. oleracea* cultivars may make them useful parents in interspecific crossing programmes to reconstitute a low glucosinolate form of *B. napus*. The low glucosinolate cultivars of *B. rapa* subsp. *oleifera*

Table 1. Glucosinolates found within the *Brassica* species analysed (see Tables 2 and 3)

Trivial name	Abbreviation	Glucosinolate side chain R (see Fig. 1)
Sinigrin	SIN	2-Propenyl
Gluconapin	GLN	3-Butenyl
Glucobrassicanapin	GLBN	4-Pentenyl
Progoitrin	PRO	2-Hydroxy-3-butenyl
Glucoiberverin	GIBV	3-Methylthiopropyl
Glucoiberin	GIB	3-Methylsulphinylpropyl
Glucoraphanin	GLR	4-Methylsulphinylbutyl
Glucobrassicin	GLB	3-Indolylmethyl
1-Methoxyglucobrassicin	MEOG	1-Methoxy-3-indolylmethyl

Table 2. Total leaf glucosinolate content and relative proportions of component glucosinolates in wild and cultivated representatives of the *B. oleracea* ($2n = 18$) group

	Total (μmol/g fr. wt)	SIN* (%)	GLN (%)	GLBN (%)	PRO (%)	GIBV (%)	GIB (%)	GLR (%)	GLB (%)	MEOG (%)
Wild										
B. oleracea										
Pop. 1 (U.K.)	9.08	0	79	0	3	0	0	0	18	0
Pop. 2 (U.K.)	9.07	14	45	37	0	0	0	0	4	0
B. montana										
Pop. 1 (Spain)	2.17	0	100	0	0	0	0	0	0	0
B. incana										
Pop. 1 (Sicily)	11.27	0	78	13	9	0	0	0	0	0
Pop. 2 (Italy)	4.37	0	37	0	3	0	5	41	12	2
Pop. 3 (Ischia)	9.54	0	94	0	4	0	0	0	3	0
B. rupestris										
Pop. 1 (Sicily)	11.88	0	0	0	0	0	100	0	0	0
B. drepanensis										
Pop. 1 (Sicily)	4.73	0	0	0	0	75	25	0	0	0
B. macrocarpa										
Pop. 1 (Egardi)	3.76	53	0	0	0	37	10	0	0	0
B. insularis										
Pop. 1 (Sardinia)	12.26	0	12	2	86	0	0	0	0	0
Pop. 2 (Tunisia)	6.86	93	0	0	0	0	0	0	0	7
Pop. 3 (Tunisia)	7.30	88	3	9	0	0	0	0	0	0
B. cretica										
Subsp. *laconica*										
Pop. 1 (Greece)	5.26	25	66	5	0	0	0	0	4	0
Pop. 2 (Greece)	5.15	85	4	9	0	0	2	0	0	0
ssp. *nivea*										
Pop. 1 (Greece)	9.70	24	35	40	0	0	0	0	1	0
Subsp. *cretica*										
Pop. 1 (Greece)	7.83	12	86	0	0	0	0	0	2	0
Pop. 2 (Greece)	5.68	0	97	1	0	0	0	0	2	0
Cultivated										
B. oleracea										
Var. *capitata*										
Cv. J. King	7.91	20	0	0	0	0	0	0	65	15
Var. *italica*										
Cv. Green Duke	1.56	1	0	39	0	0	0	48	12	0
Var. *botrytis*										
Cv. A.T.Y.R.	0.47	0	0	0	0	0	0	28	57	15
Var. *alboglabra*										
Cv. 1	1.44	0	94	6	0	0	0	0	0	0

*For explanation of abbreviations see Table 1.

Table 3. Total leaf glucosinolate content and relative proportions of component glucosinolates in wild and cultivated representatives of *B. rapa*

Subspecies	Total (μmol/g fr. wt)	SIN* (%)	GLN (%)	GLBN (%)	PRO (%)	GIBV (%)	GIB (%)	GLB (%)	MEOG (%)
Wild									
Campestris									
Pop. 1 (Algeria)	6.58	0	88	9	0	0	0	3	0
Pop. 2 (Sicily)	9.95	12	86	2	0	0	0	0	0
Pop. 3 (California)	10.15	0	95	5	0	0	0	0	0
Pop. 5 (Algeria)	6.10	1	46	53	1	0	0	0	0
Pop. 6 (Argentina)	4.15	0	95	5	0	0	0	0	0
Cultivated									
Pekenensis									
Matsushima	0.39	0	33	31	36	0	0	0	0
Japonica									
Sensujikyona	2.90	0	86	14	0	0	0	0	0
Oleifera									
Cv. 1	0.07	0	0	0	71	0	0	29	0
Cv. 2	0.45	0	0	51	49	0	0	0	0

*For explanation of abbreviations see Table 1.

may be particularly useful for this purpose and preferable to the leafy *B. rapa* subsp. *chinensis* which is currently being used as a low glucosinolate parent [15] to breed oilseed cultivars of *B. napus.*

The pattern of glucosinolates was more variable within the *B. oleracea* group than within *B. rapa.* The majority of the *B. oleracea* group possessed one major glucosinolate, with low levels of a number of others (Table 2). Geographical patterns in the distribution of glucosinolates within wild populations were evident. In the more northerly species, such as *B. incana*, *B. oleracea* and *B. montana*, gluconapin was the dominant glucosinolate, while in *B. cretica* and the Tunisian populations of *B. insularis* sinigrin occurred in high amounts. Sicilian species contained high levels of glucoiberin and glucoiberverin, while the Sardinian population of *B. insularis* contained predominantly progoitrin.

The distribution of glucosinolates within the *B. oleracea* group is of particular interest and confirms the result of a previous investigation into the occurrence of glucosinolate hydrolysis products [8] that large differences occur amongst the different taxa of this group. This previous study, however, suggested that the Sicilian populations of *B. rupestris* and *B. depranensis* had very low glucosinolate levels. However, as isothiocyanates from glucoiberin and glucoiberverin were not included in this study, the overall levels of glucosinolates would have been underestimated. The present investigation demonstrates that these species possess equivalent levels of glucosinolates to the other taxa.

Glucoiberin, the dominant glucosinolate within *B. rupestris*, is not found at high levels within any of the other wild populations, but does occur in significant amounts within cabbage and Savoy cabbage [16]. This suggests that genes from these Sicilian populations are probably present in the cultivated forms and thus supports the idea that cultivated *B. oleracea* has a multiphyletic origin [4, 11]. Likewise sinigrin, which is found within many *B. oleracea* cultivars [9, 12, 16], is restricted to the Greek and Tunisian populations, which also supports the thesis that cultivated *B. oleracea* did not just arise from western European wild forms. The occurrence of sinigrin within *B. oleracea* pop. 2 may suggest that this population is an escape from cultivation, as suggested by Mitchell [17].

The finding of Phelan and Vaughan [12] that *B. oleracea* var. *alboglabra* contains predominantly gluconapin is confirmed. This variety is considered to have been derived from the *B. cretica* complex. However, in view of the high levels of sinigrin found within most of the *B. cretica* populations (Table 2), and its absence from *B. oleracea* var. *alboglabra*, the origin of this variety needs to be reconsidered.

The very different glucosinolate contents of the Sardinian and Tunisian populations of *B. insularis* may imply that they should not be grouped together within the same species. The occurrence of sinigrin within the Tunisian populations suggests a closer affinity to the Greek *B. cretica.* It may be appropriate to return to the previous nomenclature which referred to Tunisian *B. insularis* either as *B. cretica* subsp. *atlantica* or *B. atlantica.*

In contrast to the *B. oleracea* group, wild *B. rapa* populations all had similar glucosinolate contents, comprising of mainly gluconapin (Table 3).

Our survey of glucosinolates suggests that a more extensive investigation of the occurrence and distribution of glucosinolates within and between the wild populations and species is justified. Glucosinolate patterns may provide good markers for re-evaluating the taxonomy of this complex group. The occurrence of single glucosinolates in large quantities within many of the plants examined also indicates that this material may be useful for studying the inheritance of glucosinolates.

EXPERIMENTAL

Seeds of a range of wild and cultivated *Brassica* species were obtained from seed banks (Table 4 and 5). Plants were grown in

Table 4. Sources of cultivated lines of *B. oleracea* and *B. rapa*

Cultivar	Accession number and seed bank	
B. oleracea		
Var. *alboglabra* cv 1	2921,	Universidad Politecnica, Madrid
Var. *capitata* cv January King	3350,	National Vegetable Research Station, Wellesbourne, U.K.
Var. *botrytis* cv All The Year Round	4247,	National Vegetable Research Station, Wellesbourne, U.K.
Var. *italica* cv Green Duke	—	Commercial
B. rapa		
Subsp. *japonica* cv Sensujikyona	—	Vegetable and Oriental Crops Research Station, Ano, Japan
Subsp. *pekinensis* cv Matsushima-jun	—	Vegetable and Oriental Crops Research station, Ano, Japan
Subsp. *oleifera* cv 1	019030,	Institut für Pflanzenbau und Pflanzenzüchtung, Braunschweig, F.R.G.
Subsp. *oleifera* cv 2	011008,	Institut für Pflanzenbau und Pflanzenzüchtung, Braunschweig, F.R.G.

Table 5. Source and country of origin of the different populations of *B. rapa* subsp. *campestris* and the wild representatives of the *B. oleracea* group

Origin	Accession number and seed bank	
B. rapa subsp. *campestris*		
Pop. 1 Algeria	89–06	Royal Botanic Gardens, Kew
Pop. 2 Piane degli, Sicily	6652,	Universidad Politecnica, Madrid
Pop. 3 California	1742,	Universidad Politecnica, Madrid.
Pop. 4 Don el Beide, Algeria	6464,	Universidad Politecnica, Madrid
Pop. 5 Necochea, Argentina	5903	Universidad Politecnica, Madrid
B. oleracea L.		
Pop. 1 Glamorgan, U.K.	2192,	Universidad Politecnica, Madrid
Pop. 2 Cornwall, U.K.	059–05,	Royal Botanic Gardens, Kew
B. montana Pourr.		
Pop. 1 Cape Norfeo, Spain	3607,	Universidad Politecnica, Madrid
B. incana Ten.		
Pop. 1 Cape Tindari, Sicily	3820,	Universidad Politecnica, Madrid
Pop. 2 Mt. Alburni, Italy	—	University of Lund, Sweden
Pop. 3 Is. Ischia, Italy	—	University of Lund, Sweden
B. rupestris Rafin.		
Pop. 1 Nr. Palermo, Sicily	3822,	Universidad Politecnica, Madrid
B. depranensis (Car.) Dam.		
Pop. 1 Mt. Erice, Sicily	3821,	Universidad Politecnica, Madrid
B. macrocarpa Guss.		
Pop. 1 Egardi Is., Sicily	3819,	Universidad Politecnica, Madrid
B. insularis Moris.		
Pop. 1 Cape Caccia, Sardinia	3814,	Universidad Politecnica, Madrid
Pop. 2 Mt. Bou Kourein, Tunisia	—	University of Lund, Sweden
Pop. 3 El Haouira, Tunisia	1955,	Universidad Politecnica, Madrid
B. cretica subsp. *cretica* Lam.		
Pop. 1 Euboea, Greece	0719,	Swedish University of Agricultural Science, Svalov
Pop. 2 Nr. Athens, Greece	0709,	Swedish University of Agricultural Science, Svalov
B. cretica subsp. *laconica* M. Gust. & S. Snog.		
Pop. 1 Leonidion, Greece	0712,	Swedish University of Agricultural Science, Svalov
Pop. 2 Valanidida, Greece	0714,	Swedish University of Agricultural Science, Svalov
B. cretica subsp. *nivea* M. Gust. & S. Snog.		
Pop. 1 Diakofto, Greece	0711,	Swedish University of Agricultural Science, Svalov

12 cm pots in growth chamber at 15° and a light intensity of 250 μmol quanta/m^2/sec in compost (Levingtons' Universal) for 10 weeks. From one plant of each cultivar or population, a young newly expanded leaf and an old leaf prior to senescence were sampled and glucosinolates were extracted and analysed by the method of ref. [18].

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