Genetic diversity of wild, weedy and cultivated forms of *Brassica rapa*

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Restriction Fragment Length Polymorphisms (RFLPs) were used to study the genetic diversity within and between accessions of 'wild' and cultivated *B. rapa*. Two of the wild accessions were likely to be escapes from cultivation because of their geographical origins (Argentina and California). The nature of the other three wild accessions (from Turkey, Algeria and Sicily) was not known. Principal components analysis placed the Argentinian, Californian and Turkish accessions within a cluster which contained all the cultivated forms of *B. rapa*. The other two *B. rapa* accessions were genetically divergent and, on the basis of their RFLP genotypes, would have been considered to be more distant from the cultivated forms of *B. rapa* than accessions of *B. nigra* and *B. montana*. The implications of these results for germplasm conservation, selection of material for breeding programmes and phylogenetic studies on the origin of *Brassica* crops are discussed.

Keywords: *Brassica rapa*, diversity, RFLPs, wild germplasm.

Introduction

Recent advances have been made in *Brassica* taxonomy based upon RFLPs. Comparisons between wild and cultivated accessions of *B. oleracea* L. and *B. rapa* L. have led to hypotheses concerning the site of domestication of *Brassica* crops and their subsequent spread to other geographical regions (Song *et al.*, 1988, 1992; Warwick & Black, 1991). Wild forms of both *B. oleracea* and *B. rapa* are difficult to distinguish from relatively recent escapes from cultivation (Mitchell, 1976). This has led to the inaccurate description of many accessions in germplasm collections as being 'wild', whereas they are weedy remnants of previous cultivations. This may lead to incorrect deductions concerning the origin of cultivated taxa. It may also lead to an underestimation of the relative amounts of genetic diversity within and between wild and cultivated forms of crop species which has implications for genetic conservation and the choice of genotypes for breeding programmes.

To examine how incorrect identification of *Brassica* accessions may lead to problems with deducing evolutionary relationships we obtained from germplasm collections six accessions of *B. rapa* which were described as 'wild' and compared these with cultivated

taxa (Table 1). Five of the wild accessions were inter-fertile with *B. rapa* and on the basis of their morphology, aliphatic glucosinolate content and cytology were consistent with being classified as *B. rapa* L. (R. F. Mithen and J. H. Crouch, unpublished data). A sixth 'wild' *B. rapa* accession from Egypt (UPM 4623) was found to be *B. juncea* Coss. & Czern. Of the five wild accessions, one was collected in Argentina and one in California. These are very likely to be escapes from cultivation as it is highly improbable that wild *B. rapa* occurs in the New World. The other three accessions were collected from Turkey, Sicily and Algeria. The accession from Turkey was collected in arable fields and may also be an escape from cultivation (Öztürk *et al.*, 1985). The accession from Sicily was collected from a road verge. No information is available concerning the origin of the Algerian accession.

Materials and methods

Plants were grown in John Innes No. 1 compost in insect-proof glasshouses in a 18°C day/12°C night temperature regime. Supplementary lighting was provided during the winter. Total genomic DNA preparations were made from freeze-dried leaf material of between two and five individuals from each accession. *Eco*RI-restricted DNA fragments were separated by electrophoresis and were capillary blotted

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Table 1. Origin of *Brassica* accessions used in the present study

Accession	Code no.	Genome	Origin	Description and donor number
<i>B. rapa</i> subspecies				
<i>rapifera</i>	99	A	Netherlands	Stubble turnip
<i>oleifera</i>	29	A	Germany	Oilseed rape cv. Makelsberg
<i>oleifera</i>	529	A	Canada	Oilseed rape cv. Tobin
<i>oleifera</i>	532	A	Canada	Oilseed rape cv. Parkland
<i>italica</i>	189	A		Vegetable cv. Brocoletto, HRI 6218
<i>chinensis</i>	333	A	Asia	Chinese cabbage var. chinensis, IPSR #R-C-26
<i>chinensis</i>	699	A	Asia	Chinese cabbage cv. Grannaat
<i>sylvestris</i>	73	A	Turkey	Wild, UPM 6278
<i>sylvestris</i>	74	A	Argentina	Wild, UPM 5903
<i>sylvestris</i>	75	A	Sicily	Wild, UPM 6652
<i>sylvestris</i>	76	A	Algeria	Wild, UPM 6464
<i>sylvestris</i>	79	A	California	Wild, UPM 1742
Outgroups				
<i>B. nigra</i>	179	B	Greece	Ecotype, BGRC 34180
<i>B. nigra</i>	211	B	Germany	Wild, BGRC 32960
<i>B. montana</i>	196	C	France	Wild, BOS 12

Germplasm collections prefixes: HRI: Horticulture Research International, UK; IPSR: Institute of Plant Science Research, UK; UPM: Universidad Politecnica Madrid, Spain; BGRC: Institut für Pflanssbau, Germany; BOS: Institut National de la Recherche Agronomique, France.

Table 2. List of probes used in the present study, the number of RFLPs they detected and the RFLP alleles unique to either *B. rapa* subsp. *sylvestris* no. 75 or no. 76

Probe	Polymorphic alleles	Alleles specific to	
		No. 75	No. 76
pW2E12	8	—	1
pW9A2	8	1	—
pW7B6	16	1	1
pW1F6	14	—	—
pW2B7	16	1	1
pW2A11	16	—	—
pO10	8	—	2
pO112	10	1	—
pO118	7	—	1
pR54	13	—	—
pR115	7	—	1

onto a nylon filter. Filters were probed with 11 anonymous gDNA probes derived from libraries of small (0.8–1.6 kb) *Pst*I-restricted fragments from *B. napus* L., *B. oleracea* and *B. rapa*. The probes used for detecting RFLPs are listed in Table 2. Full details of DNA extraction, blotting and Southern hybridization are provided by Magrath *et al.* (1994).

Each restriction fragment was considered as a unit character and was used to create a binary matrix. This matrix was used for principal components analyses with the aid of a program provided by M. Ambrose (John Innes Centre, Norwich) written within the GENSTAT 5 software (Rothamsted Experimental Station, England). Similarity coefficients were calculated according to the Jaccard coefficient (as described in Gower & Legendre, 1986) for all possible pairwise comparisons between genotypes (Table 3). The first and second principal components were then graphically presented (Figs 2 and 3). Similar methods of data analysis have been adopted by Wetton *et al.* (1987), Song *et al.* (1990) and Lashermes *et al.* (1993).

Results

One hundred and twenty-three restriction fragments were scored across all accessions. The *Brassica* accessions tested showed a very high level of polymorphism (Fig. 1) and no fragments were common for all accessions. The wild accessions from Sicily and Algeria contained several unique restriction fragments (Table 2). With the exception of one unique fragment which occurred in *B. rapa* subsp. *italica* no. 189, none of the cultivated subspecies or the accessions from California, Argentina and Turkey possessed any unique restriction fragments.

Table 3. Similarity matrix of *D* values for *B. nigra* (2,3), *B. montana* (1,4,31), *B. rapa* subsp. *rapifera* (5,10), European *oleifera* (6,7), Canadian *oleifera* (8,9), *italica* (17,18), *chinensis* (16,19,20) and *B. rapa* subsp. *sylvestris* accessions collected from California (11,12), Argentina (13,14,15), Turkey (21,30), Algeria (22,23,24), Sicily (25,26,27,28,29)

[illegible]

Principal components analysis clustered the accessions into several groups (Fig. 2). All seven cultivars of *B. rapa* clustered together, along with the accessions of *B. rapa* collected in Argentina and California, suggesting, as expected, that these two accessions represented weedy escapes as opposed to true wild forms of *B. rapa*. The Turkish accession of *B. rapa* was also situated close to all the cultivars. The other wild accessions from Sicily (no. 75) and Algeria (no. 76) formed two separate clusters which were more distant from the cultivated and weedy *B. rapa* accessions than the out-group represented by *B. montana* Pourr. and *B. nigra* (L.) Koch. The first principal component explained 25.5 per cent of the variation in RFLPs and separated the wild accessions from Sicily and Algeria from the other accessions. The second principal component explained 18.3 per cent of the variation and separated

the accessions from Algeria and Sicily from each other. Further statistical analysis did not significantly alter the results.

Closer examination of the principal components analysis (Fig. 3) revealed grouping of taxa within the main cluster containing the cultivated and weedy *B. rapa* accessions. The *B. rapa* subsp. *oleifera* (Metzg.) Sinsk accessions were relatively distant from the *B. rapa* subsp. *chinesis* (L.) Makino accessions, with the other weedy and cultivated taxa falling between these two groups. These results are similar to those of Song *et al.* (1990) in which Asiatic forms of *B. rapa* were shown to be phylogenetically distinct from *B. rapa* subsp. *rapifera* (Metzg.) Sinsk and subsp. *oleifera*. The *B. rapa* subsp. *oleifera* cultivars from Canada were divergent from the European cultivar (Fig. 3). Considerable variation was observed within the *B. rapa*

Fig. 1. Southern blot hybridization of *Eco*RI-restricted genomic DNA from *B. rapa*, *B. nigra* and *B. montana* out-groups probed with clone pW199.

subsp. *italica* cv. brocolleto (no. 189). Further analyses of diversity within cultivated forms of *B. rapa* require more extensive studies with a greater number of accessions.

Discussion

The principal components analysis in this study was based on the presence or absence of 123 restriction fragments. Song *et al.* (1990) concluded that the analysis of a limited number of restriction fragments (less than that used in this study) was sufficient to describe the major sources of genetic diversity between *Brassica* accessions. It is likely, therefore, that an increase in the number of restriction fragments scored (through either an increase in the number of probes or

by the use of additional restriction enzymes) would not significantly alter the conclusions. Furthermore, the probes used in this study were chosen to hybridize to fragments throughout the *B. rapa* genome (D. Lydiate, unpublished data) in order that the results would not be biased by the detection of RFLPs within selected parts of the genome, which would be particularly problematical if such regions were highly conserved. However, the very high levels of polymorphism (see Table 2) suggest that hybridization to conserved regions of the genome was not a problem. Thus, we feel that the major conclusions of this study regarding the genetic similarities and differences between the wild and cultivated accessions are valid and would not be significantly altered by increasing the number of restriction fragments detected.

Fig. 2. Plot of first and second principal components showing relationships between all accessions tested: 1, cultivated subspecies of *B. rapa*; 2, *B. rapa* subsp. *sylvestris* 79 (California); 3, *B. rapa* 74 (Argentina); 4, *B. rapa* 73 (Turkey); 5, *B. rapa* 76 (Algeria); 6, *B. rapa* 75 (Sicily); 7, *B. montana*; 8, *B. nigra*.

Fig. 3. Plot of first and second principal components showing relationships between cultivated and weedy accessions tested: *B. rapa* subspecies 1, *oleifera* (Europe); 2, *oleifera* (Canada); 3, *sylvestris* (California); 4, *sylvestris* (Argentina); 5, *rapifera*; 6, *italica*; 7, *chinensis*.

The RFLPs and principal components analyses have suggested that two accessions of *B. rapa* are genetically distant from other forms of *B. rapa*. This result is supported by a study of secondary metabolites (Rouxel *et al.*, 1991) in which both of these accessions possessed indole compounds which are not normally found within *B. rapa*. Furthermore, both accessions possess high levels of resistance to the fungal pathogen *Leptosphaeria maculans* (Desm.) Ces. et de Not. which has not previously been described in *B. rapa* (Rouxel *et*

al., 1991; Crouch *et al.*, 1994). Both accessions have been used in breeding programmes of *B. rapa* and in the development of synthetic forms of *B. napus* by interspecific hybridization with *B. oleracea* (Crouch *et al.*, 1994). These two accessions, therefore, seem to represent forms of *B. rapa* which are genetically distinct from cultivar and weedy types, and this raises questions concerning the phylogenetic deductions made in other studies which have used 'wild' forms of *B. rapa*. Sicily has been shown to be an important centre of diversity for wild C genome *Brassica* taxa (Mazzola & Raimondo, 1988; Snogerup *et al.*, 1990); this study has indicated that it may also be an important area for wild forms of *B. rapa* and suggests that further exploration is warranted.

The other three 'wild' *B. rapa* accessions clustered with the cultivated taxa. The accessions from California and Argentina are likely to be relatively recent escapes from cultivation as true wild forms of *B. rapa* are restricted to the Old World. It is probable that the Turkish accession is also a weedy escape from cultivation as opposed to a true wild form of *B. rapa*. However, results such as this have led to the suggestion that wild accessions such as the Turkish accession may represent the progenitors of cultivated forms of *B. rapa* (e.g. Song *et al.*, 1990). In these studies, it is likely that a comparison is only being made between cultivars and a weedy escape from cultivation. In addition to confusion between wild and weedy accessions, it is noteworthy that one of the 'wild *B. rapa*' accessions which was examined was in fact *B. juncea*.

In studies of crosses between wild and cultivated tomatoes, deVicente & Tanksley (1993) described transgressive segregation in an F_2 population for a number of agronomic traits. It was shown that the wild species carried alleles for several agronomically important yield characters although this was not apparent from their phenotypes. It is likely that a similar phenomenon occurs in other crop species. RFLP analyses as described in this study may provide a means to select genetically divergent wild forms of crops, such as the *B. rapa* accessions from Sicily and Algeria, in which useful alleles are present but which are not phenotypically expressed. This analysis may also provide a means to make informed choices concerning priorities for germplasm conservation and characterization.

The confusion in identification of wild *B. rapa* accessions is common to several other crop species. For example, wild forms of *Vigna unguiculata* (L.) Walp. were once only considered to occur in West Africa whereas it is now known that the greatest degree of genetic diversity in this species is actually found in wild forms which are abundant in southern Africa (Mithen & Kibblewhite, 1993; Vaillancourt *et al.*,

1993). Many of the previously described 'wild' accessions of *V. unguiculata* are naturalized cowpeas. The recent interest in the conservation of wild crop relatives has resulted in an increase in the number of accessions in gene banks which are described as being 'wild'. Many of these are likely to be recent escapes from cultivation and may represent no greater levels of genetic diversity than that of cultivated forms and will lead to an underestimation of the genetic divergence between crops and their wild relatives (e.g. Letschert & Fese, 1993). Studies on genetic diversity using RFLPs and other molecular markers may indicate wild accessions which are the most genetically divergent from cultivars and which contain unique alleles. These accessions may be the most productive material for seeking useful agronomic characters such as pest and disease resistance, particularly if the introgression of these characters into cultivars can be aided through the use of molecular markers.

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