ISSN 1479-2621
Plant Genetic Resources: Characterization and Utilization 5(3); 142–153
DOI: 10.1017/S1479262107842675

Genetic variation in *Eruca vesicaria* (L.) Cav.

S. I. Warwick[1]*, R. K. Gugel[2], C. Gómez-Campo[3] and T. James[1]

[1]*Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, K.W. Neatby Building, CEF, Ottawa, Ontario, Canada, K1A 0C6,* [2]*Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, Saskatchewan, Canada, S7N 0X2 and* [3]*Dpto. Biología Vegetal, ETSI Agrónomos, Universidad Politécnica de Madrid, 28040-Madrid, Spain*

Received 17 May 2006; Accepted 25 October 2006

Abstract

Eruca vesicaria subsp. *sativa* (syn. *E. sativa*) is a cruciferous vegetable and oilseed crop that is high in erucic acid. It occurs throughout the Mediterranean region and western Asia, and has been naturalized elsewhere as a crop/weed escape. It is closely related to subsp. *vesicaria* and subsp. *pinnatifida*, which are endemic to Spain and north-western Africa, respectively. This study evaluated patterns and levels of diversity in the three subspecies based on 234 amplified fragment length polymorphisms (AFLP), and evaluated agronomic and seed quality data in a field trial in western Canada. AFLP data revealed three main clusters: 'Sativa' (33 accessions of subsp. *sativa*), 'Vesicaria' (nine accessions of subsp. *vesicaria*) and a 'Pinnatifida' cluster (one accession of subsp. *pinnatifida* and three Moroccan accessions of subsp. *sativa*). The Sativa cluster separated into Mediterranean and Asian groups, likely reflecting differences in origin (wild versus cultivated) or primary usage, vegetable versus seed oil. The origin of the introduced Mexican population was confirmed as subsp. *sativa*. The highest levels of diversity were found in the Sativa cluster (88% AFLP polymorphisms) and the least in the Vesicaria (56%) and Pinnatifida (39%) clusters. Extensive variation was observed among the 159 subsp. *sativa* accessions evaluated in the field trial, and overall findings indicated a favourable agronomic potential.

Keywords: amplified fragment length polymorphisms; *Eruca*; genetic diversity; rocket

Introduction

The taxonomic position and ranking of the taxa named *Eruca vesicaria* (L.) Cav., *E. sativa* Miller and *E. pinnatifida* (Desf.) Pomel are controversial. Treated as three separate species by Greuter *et al.* (1986), more recently *E. vesicaria* and *E. sativa* have been united as subspecies under the older, accepted name *E. vesicaria* (Gómez-Campo, 1993, 1999; Tutin, 1993; Jalas *et al.*, 1996). Gómez-Campo (2003) concluded that although it was difficult to ascribe specimens to a particular subspecies based on morphological traits, the presence/absence of persistent sepals and the length of lower fruit pedicels were appropriate as discriminators. *Eruca pinnatifida* has long been considered a subspecies of *E. vesicaria* (Maire, 1965). For the remainder of this paper, these three taxa will be referred to as *E. vesicaria* subsp. *sativa*, subsp. *vesicaria* and subsp. *pinnatifida*.

Subspecies *sativa* is cultivated in southern Europe, north and north-east Africa, the Middle East, central Asia and north and central India. It occurs as a wild plant in the Mediterranean area, north and north-east Africa, the Balkans and the Near and Middle East to Afghanistan, but its true native range is difficult to ascertain since it has been introduced or escaped and naturalized in several European and Asian countries and even on other continents, including North and South America and Australia (Al-Shehbaz, 1985; Specht and Diederichsen, 2001). It is also a cosmopolitan weed and a host

* Corresponding author. E-mail: warwicks@agr.gc.ca

for several fungi and viruses that attack other cruciferous crops (Al-Shehbaz, 1985). Its earliest cultivation dates back to the ancient Romans and Greeks. The pods of cultivated forms are more robust and their seeds are larger than those of the wild forms (Gómez-Campo and Prakash, 1999). It is currently grown in Europe and infrequently in North America as a pungent or non-pungent salad (arugula, rocket, rúcula), spice (in sauces and prepared mustard) or medicinal plant; it is also cultivated extensively in central and western Asia for seed oil, called 'jamba-oil' in India (Al-Shehbaz, 1985; Yaniv *et al.*, 1998; Specht and Diederichsen, 2001). The oil is used for human nutrition, as lamp oil, as a lubricant, and for medicinal and cosmetic purposes (Al-Shehbaz, 1985; Yaniv *et al.*, 1998). In Asia, the seed cake and the entire plant are also used as fodder for domestic animals. The seed oil content of subsp. *sativa* ranges from 22 to 41% and is rich in erucic acid (Al-Shehbaz, 1985; Yaniv *et al.*, 1991, 1998; Yadava *et al.*, 1998; Mandal *et al.*, 2002), which makes this species a potential future source of industrial oil (Yaniv *et al.*, 1998). The seed also contains significant amounts of 4-methylthiobutyl glucosinolate (glucoerucin), which has both direct and indirect antioxidant activity and may be of value for human nutrition (Barillari *et al.*, 2005) in addition to several potential non-food uses (Tiyagi and Alam, 1995; Angelini *et al.*, 1998). Subsp. *vesicaria* from Spain is not cultivated (Gómez-Campo and Prakash, 1999), while the North African subsp. *pinnatifida*, found in eastern Morocco and central regions of Algeria and Tunisia, is sometimes cultivated as a leaf vegetable or grown as a fresh forage in oases in the Sahara (Specht and Diederichsen, 2001).

Molecular markers, including random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP) and simple sequence repeat polymorphisms (SSR), are increasingly being utilized as tools for assessing genetic diversity in crop species (Powell *et al.*, 1996). The AFLP technique produces highly reproducible, dominant markers well suited for estimating levels of genetic diversity (Vos *et al.*, 1995). The technique has been applied to assess genetic variation in various crucifer oilseeds including *Brassica carinata* A. Braun (Warwick *et al.*, 2006), *B. juncea* (Srivastava *et al.*, 2001; Burton *et al.*, 2004), *B. napus* (Lombard *et al.*, 2000; Sobotka *et al.*, 2004), *B. nigra* L. Koch (Negi *et al.*, 2004), *B. rapa* (Zhao *et al.*, 2005) and *Raphanus sativus* (Huh and Ohnishi, 2002; Muminović *et al.*, 2005). The purpose of this study was to evaluate patterns and levels of genetic diversity in the *vesicaria–sativa–pinnatifida* complex based on AFLP polymorphisms, and to evaluate agronomic and seed quality data from accessions of *E. vesicaria* subsp. *sativa* grown in the field in western Canada.

Materials and methods

Plant material

Accession numbers and sources of seed for 184 *Eruca* accessions are listed in Supplementary Table 1 (available online only at http://journals.cambridge.org). Most accessions of subsp. *sativa* were received from the United States Department of Agriculture (USDA) GRIN system (as *E. sativa* Mill.) or the Agriculture and Agri-Food Canada (AAFC) Saskatoon Research Centre (SRC). A further 14 accessions of subsp. *sativa*, nine of subsp. *vesicaria* and one of subsp. *pinnatifida*, were collected from natural habitats from various regions of Spain and Morocco; one accession of subsp. *sativa* from Mexico was obtained from the AAFC Eastern Cereal and Oilseed Research Centre (ECORC) collection (Ottawa). A sub-sample of 46 accessions was subjected to AFLP analyses (Supplementary Table 1, Fig. 1); including representatives from all three subspecies, with accessions of subsp. *sativa* chosen to represent a broad range of geographic diversity for origin, including Afghanistan, Cyprus, Egypt, India, Iran, Morocco, Pakistan, Spain and Turkey. For the field trials, 159 accessions of subsp. *sativa* from the USDA and the AAFC-SRC were tested.

AFLP protocols

Plants were grown in a controlled environment. Two to three young leaves were collected from each of 10 individuals per accession and stored at −80°C; two bulked samples per accession (b1 and b2) were prepared with equal amounts of leaf tissue from five individuals. Genomic DNA was extracted from lyophilized and ground material using the Nucleon PhytoPure DNA Isolation Kit (GE Healthcare Bio-Sciences Corp., Piscataway, New Jersey, USA) according to the manufacturer's directions. All bulked DNA samples were diluted to 50 ng/μl. AFLPs were generated based on the protocol of Vos *et al.* (1995) as detailed in Warwick *et al.* (2006). Four selective primer pairs were used (Table 1). The amplified products were separated by polyacrylamide gel electrophoresis (PAGE) in an automated sequencer (LI-COR, Lincoln, Nebraska, USA) and infrared gel images analysed using a GeneIR (Scanalytics) program.

Field trials

The 159 subsp. *sativa* accessions (Supplementary Table 1, Fig. 2) were sown in mid-May at the AAFC-SRC Research Farm, Saskatoon, Saskatchewan, Canada (52°07′N 106°38′W) in 1997 and 1998. Each plot consisted of two rows, 18 cm apart and 3 m in length with 100 seeds per row. Plots were 1 m apart and flanked by two rows of

https://doi.org/10.1017/S1479262107842675 Published online by Cambridge University Press

barley. Limited seed availability in 1997 allowed only single plots of each accession to be sown that year; in 1998, two replicate plots of each accession were sown using seed harvested from isolation plots grown in 1997. Agronomic and seed quality traits of interest to plant breeders were evaluated on a plot basis as follows:

- Days to flower: the number of days from seeding to when about 20% of the plants had open flowers.
- Days to maturity: the number of days from seeding to when about 90% of the pods had changed colour and seeds were firm, equivalent to a moisture content of about 25%.
- Crop height: the mean of two measurements taken near the centre of each plot after flowering and prior to swathing maturity.
- Lodging severity was determined after flowering and prior to swathing maturity using a visual rating scale of 1 (plants erect) to 5 (plants flat on ground).
- Blackleg severity was evaluated on plants at swathing maturity in a field where disease inoculum was present. Westar, a *B. napus* cultivar highly susceptible to blackleg, was sown in single rows to provide and monitor disease pressure. A random sample of 25 plants per accession per replicate (one replicate in 1997; two replicates in 1998) was uprooted; each plant was cut through the hypocotyl and/or tap root and scored for blackleg severity using a rating scale of 0 (no diseased tissue visible in the cross-section) to 5 (diseased tissue occupies 100% of cross-section with significant constriction of affected tissues; plant dead). Mean blackleg severity over all replicates was calculated for each accession.
- Seed weight: a random sample of 500 seeds was dried to $<5\%$ moisture content and weighed.
- Oil content was determined using continuous wave, low-resolution nuclear magnetic resonance (AOCS, 2000).
- Protein content was analysed by the Dumas Total Combustion Method (AOCS, 1999).
- Fatty acid composition of the seed oil was determined by gas chromatography (AOCS, 1997) following preparation of fatty acid methyl esters by base-catalysed methanolysis (Thies, 1971).
- Glucosinolate composition and content of the seed meal were determined by gas chromatography (Sosulski and Dabrowski, 1984).

Data analysis

AFLP bands were scored as either present (1) or absent (0) for all samples. Fragments showing the same electrophoretic mobility were treated as identical. Pairwise genetic similarity measures between each accession were obtained from the binary matrix with the Numerical Taxonomy and Multivariate Analysis package in NTSYSpc version 2.02 (Rohlf, 1997) using the SIMQUAL option. The Sokal and Michener's simple matching (SM) coefficient and the Jaccard (J) coefficient were computed. Dendrograms were generated from each similarity matrix using the unweighted pair-group arithmetic average (UPGMA) clustering procedure in a sequential agglomerative hierarchical combinatorial (SAHN) strategy. The degree of relationship between the two similarity matrices (SM and J) was measured using the product–moment correlation r [= normalized Mantel statistic Z (Mantel, 1967)] between the two similarity matrices calculated using the MXCOMP function in NTSYS. A cophenetic correlation procedure was also used to compare the matrix of ultrametric distances (generated from the tree matrix) with the pairwise similarity matrix. An analysis of molecular variance (AMOVA) was performed using Arlequin version 3.01 (Excoffier *et al.*, 2006) to partition the total genetic variation among and within the clusters observed in the UPGMA analysis. The ordination method principal components analysis (PCA), available in NTSYS, was also used to confirm the results of cluster analysis (data not shown).

Means, standard deviations and ranges were calculated in Microsoft Excel for each trait of the 159 subsp. *sativa* accessions (see Table 2). The data, with the exception of 'Other glucosinolates', 'Other fatty acids', 'Total saturated fatty acids' (excluded as minor components) and 'Total glucosinolates' (excluded as a derived value), were analysed using NTSYS. The data were subjected to linear transformation using the STAND option to reduce the effects of different scales of measurement for the different characters. Pairwise distances between accessions were then calculated using the SIMINT option in NTSYS. Two distance coefficients were compared including DIST, the average taxonomic distance and EUCLID, the Euclidean distance. Both coefficients yielded identical matrices. Dendrograms were generated from the DIST matrix using the UPGMA clustering procedure in the SAHN strategy in NTSYS. A cophenetic correlation procedure was used to compare the matrix of ultrametric distances (generated from the tree matrix) with the pairwise similarity matrix. The results of cluster analysis and the relative importance of the 14 variables in separating the accessions were confirmed with PCA. The agronomic data were standardized (STAND) and a correlation matrix among the variables computed (SIMINT, CORR). The first three principal component axes (i.e. eigenvectors) were extracted from the correlation matrix (EIGEN) and a visual three-dimensional

https://doi.org/10.1017/S1479262107842675 Published online by Cambridge University Press

plot of variables defining these axes produced. Means and standard deviations were calculated for each of the resultant clusters.

Results

AFLP analysis

A total of 234 polymorphic AFLP fragments were generated [Table 1, Supplementary Table 2 (available online only at http://journals.cambridge.org)]. For each accession, the data from the two bulked samples (b1 and b2) were combined to give a single score of fragment presence/absence in a given accession. Fragment size ranged from 54 to 357 bp (Supplementary Table 2). About 20% of the 234 markers occurred at greater than 80% frequency (i.e. in 37 of the 46 accessions), while less than 10% of the markers were found in a single accession. A comparable number of markers (52–70) were generated per primer pair.

The distance matrices based on the SM and J similarity coefficients were highly correlated ($r = 0.943$). The high correlation between the SM- and J-based matrices showed that the allelic relationship between the absence and presence of a given band can be assumed (Lombard *et al.*, 2000). Since the cophenetic value for the similarity matrices generated for the SM and J coefficients was so high, only the SM data were used to generate the dendrograms presented here. A single dendrogram (Fig. 1) was obtained using the SM coefficient for pairwise distance calculations between accessions. A single dendrogram was also obtained with the J coefficient (data not shown, but available upon request from the senior author). The cophenetic correlation was also high ($r = 0.955$ and 0.922) for the SM and J coefficient matrix and the cluster matrix, indicating a good fit of the cluster analysis to the SM and J similarity matrix, respectively. Both analyses revealed four main clusters: 'Sativa' (Mediterranean + Mexico, 18 accessions of subsp. *sativa*), 'Sativa' (Asia, 15 accessions of subsp. *sativa*), 'Vesicaria' (Spain, nine accessions of subsp. *vesicaria*) and a Moroccan 'Pinnatifida' cluster (four accessions, including one accession of subsp. *pinnatifida* and three accessions of subsp. *sativa* from Morocco). Within the Sativa cluster, several accessions clustered by country of origin, the most notable being those from Spain and those from India/Pakistan. In contrast, one accession from Turkey was in the Mediterranean and the other in the Asian cluster. AMOVA (Supplementary Table 3, available online only at http://journals.cambridge.org) and PCA (data not shown) of the molecular data also revealed the same four clusters. For the AMOVA analysis, the accessions were placed in groups corresponding to either three (Sativa, Vesicaria, Pinnatifida) or four (Sativa – Mediterranean, Sativa – Asian, Vesicaria, Pinnatifida) clusters. Both AMOVA results indicated significant differences among the groups ($P = 0.03$).

Similarity values based on the SM coefficient averaged 0.705 (0.562–0.915) among the 33 accessions of the Sativa cluster, with a slightly lower mean and greater range among the 18 Mediterranean (+Mexico) accessions (0.733; 0.653–0.858) versus the 13 Asian accessions (0.823; 0.735–0.897). Similarity values among accessions within the Moroccan Pinnatifida cluster averaged 0.697 (0.650–0.722), while accessions in the Vesicaria cluster were the most similar (0.850; 0.803–0.931).

Table 1. Number of markers detected for each of four AFLP primer pairs and numbers and frequencies of polymorphic markers detected within the clusters observed in Fig. 1: Sativa (Mediterranean: 18 accessions of subsp. *sativa*; Asian: 15 accessions of subsp. *sativa*; All Sativa: 33 accessions of subsp. *sativa*, excluding three from Morocco); Vesicaria (Spain: nine accessions of subsp. *vesicaria*); and Pinnatifida (Morocco: four accessions, including one of subsp. *pinnatifida* and three of subsp. *sativa* from Morocco)

		Polymorphic markers									
		Sativa						Vesicaria		Pinnatifida	
		Mediterranean		Asian		All		Spain		Morocco	
AFLP primer pair[a]	Total No.	No.	%	No.	%	No.	%	No.	%	No.	%
1. *Eco*R1 + AGC / *Mse*1 + CAC	70	58	83	43	61	64	91	43	61	18	26
2. *Eco*R1 + AAG / *Mse*1 + CAG	59	43	73	24	41	47	80	36	61	27	46
3. *Eco*R1 + AAG / *Mse*1 + CAT	53	41	77	35	66	48	91	30	57	25	47
4. *Eco*R1 + ACC / *Mse*1 + CTT	52	43	83	27	52	48	92	22	42	21	40
Total	234	185	79	129	55	207	88	131	56	91	39

[a] The core sequences of primers for the selective amplification were as follows: 5′GACTGCGTACCAATTC3′ for the *Eco*R1 primer and 5′GATGAGTCCTGAGTAA3′ for the *Mse*1 primer. Each primer contained three selective nucleotides at the 3′ end (e.g. *Eco*R1 + AGC contained the core sequence plus AGC at the end).

https://doi.org/10.1017/S1479262107842675 Published online by Cambridge University Press

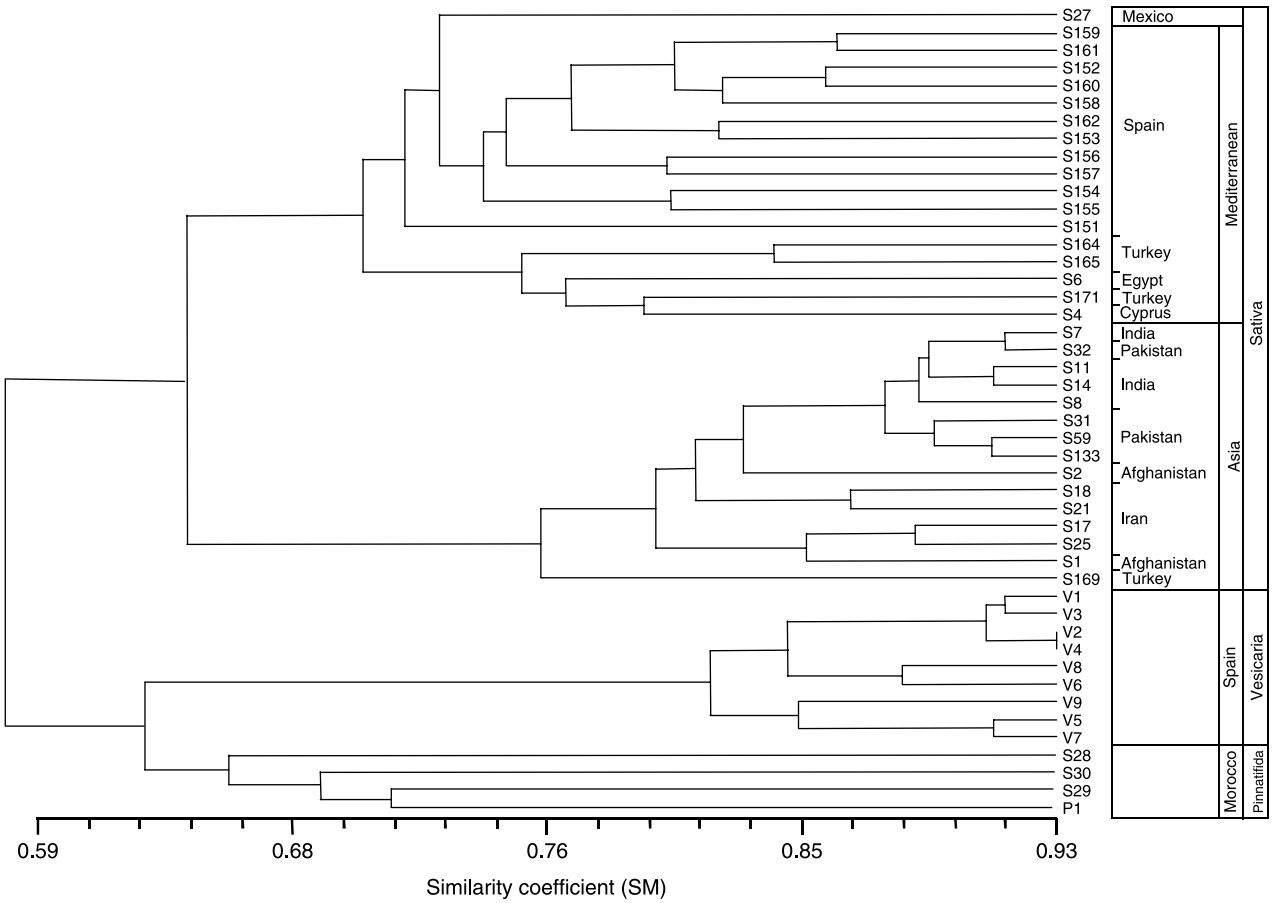

Fig. 1. Genetic similarity among 46 accessions of *Eruca vesicaria* based on AFLP data. The dendrogram was produced using UPGMA clustering of pairwise similarity distances between accessions. Accession numbers are given in Supplementary Table 1 (available online only at http://journals.cambridge.org).

The three subspecies varied in diversity levels, with accessions in the Sativa cluster having the greatest percentage of polymorphic loci (88%) compared with the Vesicaria (56%) and Pinnatifida (39%) clusters (Table 1). Differences were also detected between the two Sativa groups, with greater diversity in the Mediterranean (79%) versus Asian (55%) accessions. Levels of diversity can also be assessed by numbers of unique alleles (Supplementary Table 2). The Sativa accessions were the most diverse, with 39 of the 234 AFLP markers unique to the Sativa cluster; 11 of these were confined to the Mediterranean accessions, whereas only one was unique to the Asian accessions. Eight AFLP markers were unique to the Pinnatifida cluster, while the Vesicaria cluster had only one unique AFLP marker. The genetic variation within and among the clusters was further examined by performing AMOVA on the molecular data (Supplementary Table 3). Accessions were placed in groups corresponding to either three (Sativa, Vesicaria, Pinnatifida) or four (Sativa – Mediterranean, Sativa – Asian, Vesicaria, Pinnatifida) clusters. Among-group variation accounted for approximately 32 and 37% of the total variation in the two analyses, respectively, and among accessions within-group variation accounted for 37 and 29% of the total variation, respectively. The discriminating power of each primer pair was high, as each primer pair analysed separately was able to uniquely identify all 46 accessions tested.

Agronomic traits

Means and ranges of values for agronomic and seed quality traits, averaged for years 1997 and 1998, are given in Table 2. In general, considerable variation was observed for most traits. Individual ANOVA performed on each of the variables indicated statistically significant differences among accessions ($P < 0.05$) for all traits except 'Days to maturity' and 'Other glucosinolates'.

The among-accession pairwise similarity matrices generated for the DIST and EUCLID coefficients were identical ($r = 1.00$), and only the DIST matrix was used for

https://doi.org/10.1017/S1479262107842675 Published online by Cambridge University Press

Table 2. Means, standard deviation (SD) and ranges of values for agronomic and seed quality traits of 159 accessions of *Eruca vesicaria* subsp. *sativa* grown in field trials at Saskatoon, Saskatchewan, averaged for 1997 and 1998

Trait	Mean	SD	Range
Days to flower	41	1.8	38–48
Days to maturity	101	2.4	93–109
Crop height (cm)	88	6.9	71–120
Lodging severity (1–5)	1.6	0.5	1.0–3.0
Blackleg severity (0–5)	1.3	0.4	0.3–2.3
Thousand seed weight (g)	2.7	0.5	1.5–3.6
Oil content (% whole seed, dry wt basis)	28.4	1.1	25.1–31.3
Protein content (% whole seed, dry wt basis)	37.3	1.3	33.7–41.6
Glucosinolates (μmol/g whole seed)			
Total	135.4	6.8	110.9–152.6
4-methylthiobutyl	132.6	6.5	109.4–149.2
Other glucosinolates[a]	2.8	0.8	1.4–6.5
Fatty acids (% of total)			
C18:1 - oleic	16.1	1.4	11.6–19.0
C18:2 - linoleic	8.5	0.4	7.6–9.9
C18:3 - linolenic	10.7	0.8	9.5–13.6
C20:1 - eicosenoic	9.8	0.8	7.4–11.3
C22:1 - erucic	43.9	1.3	40.4–47.6
Other fatty acids[b]	3.7	0.3	3.1–4.9
Total saturated fatty acids[c]	7.2	0.3	6.4–8.2

[a] Includes 2-propenyl, 3-butenyl, 4-pentenyl, 2-hydroxy-3-butenyl, 2-hydroxy-4-pentenyl, 2-phenylethyl, 3-methylthiopropyl, 5-methylthiopentyl, 4-hydroxybenzyl, 3-indolylmethyl, 4-hydroxy-3-indolylmethyl and other unidentified glucosinolates.
[b] Includes C16:1 (palmitoleic), C20:2 (eicosadienoic), C22:2 (docosadienoic), C24:1 (nervonic) and other unidentified fatty acids.
[c] Includes C14:0 (myristic), C16:0 (palmitic), C18:0 (stearic), C20:0 (arachidic), C22:0 (behenic) and C24:0 (lignoceric).

generating the dendrograms presented here. The cophenetic correlation was high ($r = 0.86$) for the DIST pairwise similarity matrix and the cluster matrix, indicating a good fit of the cluster analysis to the pairwise DIST matrix. A single dendrogram (Fig. 2) was generated using the UPGMA clustering procedure. Six main clusters were evident; two clusters were represented by one or two accessions and one cluster contained the majority (123) of the accessions. Accessions did not group by geographic origin.

Principal components analysis, often used to determine the relative importance of classification variables, constructs a new set of orthogonal coordinate axes, such that the projection of points onto the axes have maximum variance. In our trials, the first three principal components explained 66.4% of the total variation in the data: 43.4% in the first, 12.5% in the second and 10.5% in the third (Supplementary Table 4, available online only at http://journals.cambridge.org). Five principal components explained 81.5% of the total variation. In the first principal component, high loadings were estimated for most traits; few traits had high loadings in the second and third principal components (Supplementary Table 4). Traits with larger loadings (+ or −) contribute proportionally more towards explaining the total variation accounted for in that particular eigenvector. Loadings associated with agronomic traits were similar in magnitude to those associated with seed quality traits. No single trait clearly dominated in separating the accessions into clusters. Cluster means for each of the 14 traits are given in Table 3. Cluster 6, consisting of a single accession (S10), was the most distinct as it was early to flower and mature, short in stature, large seeded with a high oil content, and had high proportions of oleic and eicosenoic acid.

Differences were also detected between the Mediterranean and Asian groups. Thirteen Mediterranean accessions (103 d to mature, 96 cm tall, 28.0% oil, 2.3 g/1000 seeds) were compared with 141 Asian accessions (100 d to mature, 87 cm tall, 28.5% oil, 2.8 g/1000 seeds); differences between the groups were statistically significant for crop maturity, height and thousand seed weight, but not for oil content (least significant difference, $P = 0.05$).

Discussion

AFLP analysis

The results of this study confirmed the separation of subspecies *sativa*, *vesicaria* (native to Spain) and *pinnatifida* (native to North Africa) and clearly showed that the three accessions of *sativa* from Morocco are closer to

https://doi.org/10.1017/S1479262107842675 Published online by Cambridge University Press

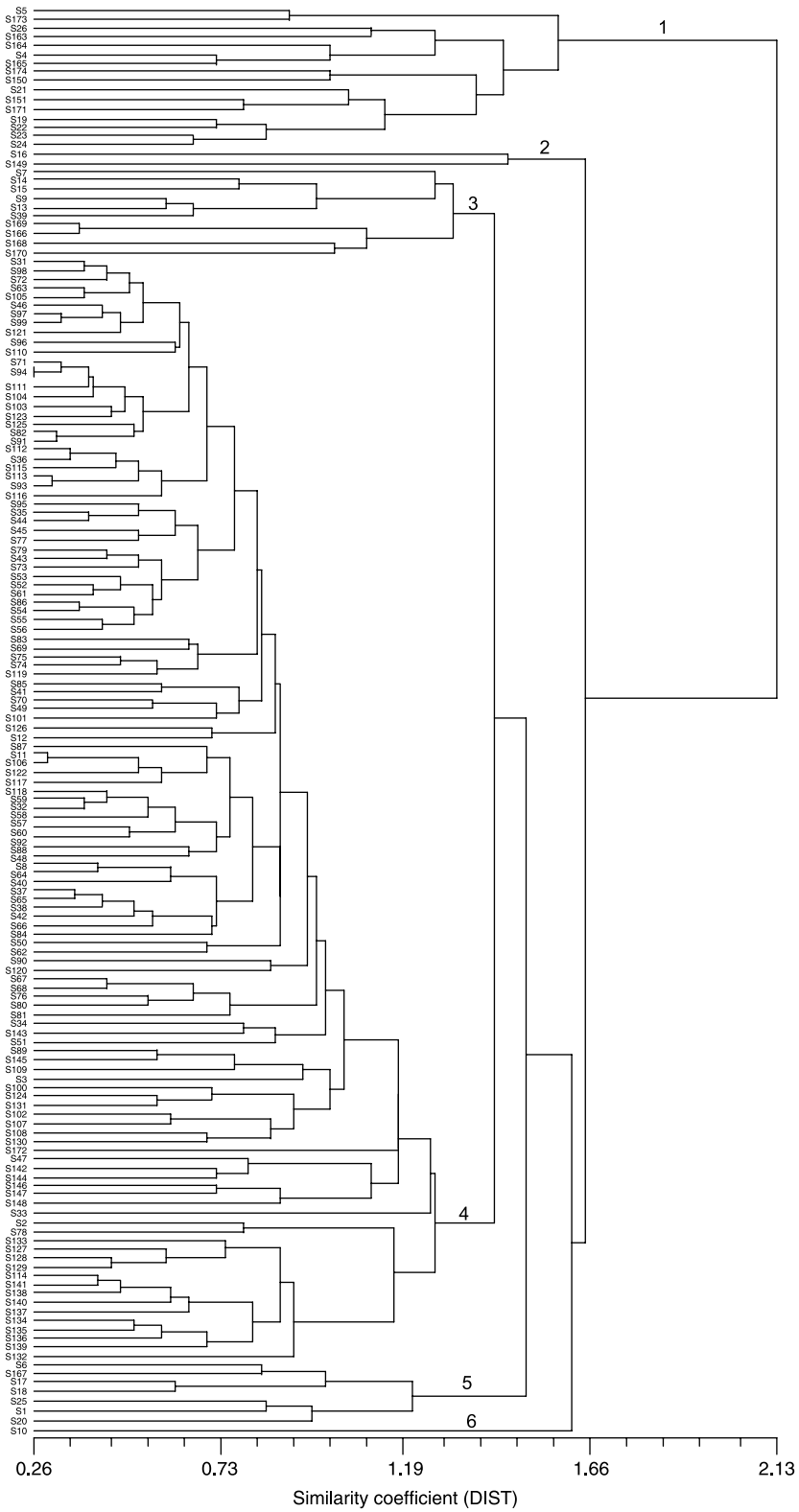

Fig. 2. Similarity among 159 accessions of *Eruca vesicaria* subsp. *sativa* based on 1997 and 1998 field evaluation data collected at Saskatoon, Saskatchewan, Canada. The dendrogram was produced using UPGMA clustering of pairwise similarity distances between accessions. Accession numbers are given in Supplementary Table 1 (available online only at http://journals.cambridge.org).

https://doi.org/10.1017/S1479262107842675 Published online by Cambridge University Press

Table 3. Means ± standard deviation by cluster for 14 traits recorded for 159 accessions of *Eruca vesicaria* subsp. *sativa* in field trials at Saskatoon, Saskatchewan, in 1997 and 1998

	Cluster[a] (No. of accessions)					
Trait[b]	1 (16)	2 (2)	3 (10)	4 (123)	5 (7)	6 (1)
DTF	45.0 ± 2.0	44.5 ± 2.1	40.5 ± 1.4	41.1 ± 1.1	42.4 ± 1.4	38.8
DTM	101.1 ± 3.5	95.3 ± 1.8	104.3 ± 1.3	100.3 ± 1.9	103.9 ± 1.6	98.5
CH	101.3 ± 7.7	76.3 ± 6.0	82.9 ± 2.9	86.7 ± 4.2	95.8 ± 3.6	70.8
LS	1.5 ± 1.5	1.0 ± 0.0	2.5 ± 0.4	1.6 ± 0.4	1.9 ± 0.2	2.0
BS	0.8 ± 0.4	0.9 ± 0.2	1.1 ± 0.3	1.4 ± 0.4	0.8 ± 0.2	2.3
SW	2.1 ± 0.4	2.1 ± 0.9	3.2 ± 0.3	2.8 ± 0.4	2.7 ± 0.5	3.3
OIL	26.8 ± 1.0	26.4 ± 0.7	29.0 ± 1.0	28.5 ± 0.9	29.4 ± 0.9	30.1
PRO	39.7 ± 1.3	37.6 ± 0.7	36.7 ± 1.3	37.1 ± 1.0	36.4 ± 1.4	37.4
MTB	130.9 ± 5.9	129.1 ± 4.8	126.2 ± 9.1	134.2 ± 4.9	118.4 ± 6.1	130.1
C18:1	12.9 ± 0.7	16.5 ± 1.0	17.9 ± 0.6	16.4 ± 0.8	15.3 ± 1.1	19.0
C18:2	8.9 ± 0.6	8.8 ± 0.4	8.2 ± 0.4	8.5 ± 0.3	8.7 ± 0.4	8.1
C18:3	12.6 ± 0.5	10.0 ± 0.1	10.6 ± 0.8	10.5 ± 0.4	11.6 ± 0.5	9.8
C20:1	8.1 ± 0.4	10.4 ± 1.0	10.3 ± 0.6	10.0 ± 0.4	9.3 ± 0.4	11.3
C22:1	46.0 ± 1.2	43.0 ± 2.3	42.4 ± 1.0	43.7 ± 0.9	44.5 ± 1.2	41.6

[a] Clusters based on UPGMA clustering of pairwise similarity distances between accessions (Fig. 2).
[b] DTF, days to flower; DTM, days to mature; CH, crop height (cm); LS, lodging severity (1–5); BS, blackleg severity (0–5); SW, thousand seed weight (g); OIL, % seed oil; PRO, % seed protein; MTB, 4-methylthiobutyl glucosinolate (μmol/g whole seed); Fatty acids (% of total): C18:1, oleic; C18:2, linoleic; C18:3, linolenic; C20:1, eicosenoic; C22:1, erucic.

pinnatifida, and that the Spanish native *vesicaria* was more similar to *pinnatifida* than to *sativa*. The results also indicated a separation of Mediterranean and Asian subsp. *sativa* accessions, which likely reflects differences in their wild or cultivated condition or perhaps in their primary usage, vegetable versus seed oil, in these two regions. The introduced weedy subsp. *sativa* collection from Mexico was confirmed as Mediterranean (most likely from Spain) in origin, consistent with the suggestion by Rollins (1993) that subsp. *sativa* is the adventive form in North America and not *vesicaria*. Indeed, subsp. *sativa* is an especially abundant and widespread weed in central and southern Mexico, where it commonly forms solid stands covering many hectares (Rollins, 1993). The three subspecies are weedy in the west Mediterranean region, but only subsp. *sativa* has spread to other regions, either as a crop or as a weed.

The level of AFLP polymorphism observed in the subsp. *sativa* accessions was as high or higher than those observed in some *Brassica* oilseed crops: 79% polymorphism in 426 AFLP markers for 18 accessions of *B. nigra* (Negi *et al*., 2004), 91% polymorphism in 524 markers for 161 accessions of *B. rapa* (Zhao *et al*., 2005), 62% polymorphism in 1251 markers for 30 lines of *B. juncea* (Srivastava *et al*., 2001), 35% polymorphism in 751 markers for 92 *B. juncea* breeding lines (Burton *et al*., 2004) and 23% polymorphism in 296 markers for 66 accessions of *B. carinata* (Warwick *et al*., 2006). The four *Eco*R1 or *Mse*1 primer pairs showed similar amounts of polymorphisms for the Sativa accessions, ranging from 80 to 92%. Similar AFLP polymorphism frequencies among primer pairs have been reported in other *Brassica* studies, including a *B. napus* study with 17 primer pairs (Lombard *et al*., 2000), a *B. juncea* study with 10 primer pairs (Burton *et al*., 2004) and a *B. carinata* study with four primer pairs (Warwick *et al*., 2006).

Information on genetic diversity and/or genetic relatedness among genotypes of the *E. vesicaria* subspecies is currently limited. The present study demonstrated the utility of AFLP markers in assessing genetic relationships between cultivars relative to their genetic origin, and was certainly effective at the regional level. AFLPs also are important markers for fingerprinting cultivars and may be useful in distinguishing *Eruca* accessions. Each primer pair in the present study showed high discriminating power, and was able to uniquely identify all 46 accessions tested. Lombard *et al*. (2000) showed that AFLP markers had high discriminating power to easily identify cultivars of *B. napus* for plant registration and protection purposes, as did Warwick *et al*. (2006) for *B. carinata*. AFLP markers could also serve as a valuable breeding tool, with genetic distance information used to facilitate the identification of diverse parents to cross in hybrid combinations in order to maximize heterosis (Charcosset and Moreau, 2004). Information on genetic relatedness can also be used for germplasm conservation and management of genetic resources, including identification of core collections.

https://doi.org/10.1017/S1479262107842675 Published online by Cambridge University Press

Agronomic traits

In general, considerable variation was observed for most agronomic and seed quality traits in this study. Unlike the results of the AFLP analysis, accessions did not group by geographic origin and no single trait clearly dominated in separating the accessions into clusters. Divergent genotypes, as identified by their assignment to different clusters, may have good breeding value and maximum variability may be achieved by utilizing such genotypes for crosses. Selection from the Asian group is likely the most appropriate for initial development of Canadian oilseed lines, as Asian accessions tended to have higher oil contents, were earlier maturing, shorter and had larger seed.

Variability in agronomic and seed quality traits has been reported previously for *E. vesicaria* subsp. *sativa*. Many of these studies were conducted with relatively few accessions grown in one (Yaniv *et al.*, 1998; Mandal *et al.*, 2002) or several (Singh and Rajput, 1993a, b; Gurgar *et al.*, 1999) environments, or with seed collected from several sites (Yaniv *et al.*, 1995). Given the significant effects of environment and genotype × environment on the expression of agronomic and seed quality traits in this species (Singh and Rajput, 1993a, b; Gurgar *et al.*, 1999), it is inappropriate to draw broad conclusions regarding species diversity from trials with few accessions. A larger number of accessions was evaluated by Sodani *et al.* (1990), who grouped 99 accessions into 13 clusters based primarily on yield components; geographic origin was not correlated with assignment of accessions to a cluster. Most (69) accessions were grouped in a single cluster and a comparison of cluster means for different agronomic and seed quality traits indicated differences in maturity, plant morphology, yield components and oil content. Yadava *et al.* (1998) evaluated 100 accessions and reported large variations in oil content and fatty acid profile including erucic, oleic, linoleic, linolenic and eicosenoic acid.

All the accessions evaluated in this study took fewer days to flower (38–48 d) and mature (93–109 d) than reported for other accessions evaluated in India (Sodani *et al.*, 1990) (51–56 d to 50% flowering, 119–124 d to mature) and Israel (Yaniv *et al.*, 1998) (60–88 d to flower, 145–163 d to mature), although the study with the fewest (10) accessions (Yaniv *et al.*, 1998) had the most variability for these traits. Most accessions in our study were also taller, and height (71–120 cm) and seed weight (1.5–3.6 g/1000) were more variable, than reported by Sodani *et al.* (1990) (57–84 cm tall, 2.3–3.9 g/1000 seeds) and Yaniv *et al.* (1998) (1.3–1.9 g/1000 seeds). Seed oil contents in our study (25–31%) were similar to those reported by Yaniv *et al.* (1998) (25–29%) and Mandal *et al.* (2002) (24–30%), but lower than those reported by Sodani *et al.* (1990) (32–36%), Yadava *et al.* (1998) (32–41%), Gurgar *et al.* (1999) (26–39%) and Sun *et al.* (2004) (23–38%). Seed protein contents in our study ranged from 34–42% and were slightly higher than those reported by Sun *et al.* (2004) (30–38%).

Large variations in fatty acid profiles among accessions were also revealed in our study. Erucic acid was the major fatty acid found in seed oil of all accessions, ranging from 40 to 48% of the total fatty acids. Oleic (12–19%), linoleic (8–10%), linolenic (9–14%) and eicosenoic (7–11%) acids were also present in significant amounts. Values reported in other studies (Yaniv *et al.*, 1991; Das *et al.*, 2001; Mandal *et al.*, 2002) generally fell within these ranges, but significantly more variation in erucic acid content was reported by Yadava *et al.* (1998) (27–52%) and Yaniv *et al.* (1998) (33–45%). Erucic acid contributes to the unpalatable flavour of the seed oil and is linked to animal health problems (Yadava *et al.*, 1998; Das *et al.*, 2001).

The primary glucosinolate in seeds of the accessions evaluated in our study was 4-methylthiobutyl glucosinolate, which comprised about 98% of the total glucosinolates and generally agreed with previous reports (Das *et al.*, 2001, 2003; Barillari *et al.*, 2005). In subsp. *sativa*, 4-methylthiobutyl glucosinolate is responsible for the pungent flavour of pressed oil-cake (Das *et al.*, 2001); in leaves, however, 4-mercaptobutyl glucosinolate predominates and likely contributes significantly to their unique flavour (Bennett *et al.*, 2002). Many glucosinolates reportedly have beneficial effects in human and animal nutrition, but the presence of glucosinolates in seed meal (oil-cake) generally has a negative effect on palatability and health when consumed in high concentrations (Rosa, 1999). The continuing practice of feeding *Eruca* oil-cake to animals (Al-Shehbaz, 1985; Das *et al.*, 2003), and its potential use in fish diets (Fagbenro, 2004), suggests a need for research to reduce glucosinolate levels in this crop.

Accessions of *E. vesicaria* subsp. *sativa* that are resistant (Tewari *et al.*, 1996) and susceptible (Li *et al.*, 2005) to blackleg have been reported. Blackleg disease pressure was low in both years of our study and mean blackleg severity values for *B. napus* cv. Westar (susceptible check; data not shown) ranged from 2.2 to 2.3 on a scale of 0–5, which is too low to identify resistant germplasm conclusively. Almost all (98%) of the accessions tested in our trials were less susceptible to blackleg than Westar and the remainder were similar to Westar. A random selection of plants of both species that had symptoms of blackleg yielded identical colonies of *Phoma lingam* (Tode ex Fr.) Desmaz., the anamorph of *Leptosphaeria maculans*, confirming that infection by the blackleg fungus did occur in *E. vesicaria* subsp. *sativa*.

The successful cultivation of a large number of *E. vesicaria* subsp. *sativa* accessions in our study suggests that this species could be grown as an oilseed crop in western Canada. There is good potential for crop improvement, as the species is reported to have tolerance to cold (as seedlings), heat, drought (Sun *et al.*, 2004) and salt (Ashraf, 1994), resistance to various pests (Singh *et al.*, 1994; Rana *et al.*, 1995; Curto *et al.*, 2005) and diseases (Tewari and Conn, 1993; Tewari *et al.*, 1996; Bansal *et al.*, 1997; Singh and Kolte, 1999), and male-sterility (Matsuzawa *et al.*, 1999) and self-incompatibility alleles for developing hybrid production systems (Verma *et al.*, 1977; Sun *et al.*, 2005). Developing subsp. *sativa* cultivars with higher yield and seed oil content (25–31%, this study) is also necessary to be competitive with existing crops that have much higher oil contents (rapeseed: 40–45%; flax: 35–45%) (Lühs and Friedt, 1994) and with other potential new oilseed crops currently under investigation, including *B. carinata* (25–36%) (Warwick *et al.*, 2006) and *Crambe abyssinica* Hochst. ex R.E. Fries (31–39%) (Warwick and Gugel, 2003). Sustainable markets for the oil and meal must be developed and could include both edible and industrial products. Erucic acid and glucosinolate levels would need to be reduced significantly to make the oil and meal acceptable for human and animal nutrition; similar breeding efforts were successful in developing canola from rapeseed (Lühs and Friedt, 1994). Conversely, breeding efforts may be needed to increase the levels of some antinutritive compounds in order to make subsp. *sativa* more attractive as an industrial crop. The oil has high repellent activity against some insects that infest stored grain and could replace more toxic pesticides for this purpose (Mohiuddin *et al.*, 1990). The meal has potential use as a low environmental impact soil fumigant to suppress soilborne plant pathogenic fungi, nematodes (Tiyagi and Alam, 1995) and germination of weed seeds (Angelini *et al.*, 1998). Such soil fumigant properties could also be improved by developing genotypes with even higher levels of glucosinolates.

Acknowledgements

Assistance with the field trials was provided by Z. Bainas and D. Hennigan, AAFC-SRC. We gratefully acknowledge Dr J. P. Raney and his technical staff, AAFC-SRC, for conducting the seed quality analyses. We also thank Drs E. Small and J. Cayouette, AAFC-ECORC, and Dr F. Katepa-Mupondwa, AAFC-SRC, for providing helpful comments on an earlier draft of this manuscript.

References

Al-Shehbaz IA (1985) The genera of Brassiceae (Cruciferae; Brassicaceae) in the southeastern United States. *Journal of the Arnold Arboretum Harvard University* 66: 279–351.

Angelini L, Lazzeri L, Galletti S, Cozzani A, Macchia M and Palmieri S (1998) Antigerminative activity of three glucosinolate-derived products generated by myrosinase hydrolysis. *Seed Science and Technology* 26: 771–780.

AOCS (American Oil Chemist Society) (1997) Determination of fatty acids in edible oils and fats by capillary GLC. *AOCS Official Method Ce 1e-91*. Champaign, Illinois: AOCS.

AOCS (American Oil Chemist Society) (1999) Generic combustion method for determination of crude protein. *AOCS Official Method Ba 4e-93*. Champaign, Illinois: AOCS.

AOCS (American Oil Chemist Society) (2000) Oil content of oilseeds by nuclear magnetic resonance. *AOCS Recommended Practice Ak 3-94*. Champaign, Illinois: AOCS.

Ashraf M (1994) Organic substances responsible for salt tolerance in *Eruca sativa*. *Biologia Plantarum* 36: 255–259.

Bansal VK, Tewari JP, Tewari I, Gómez-Campo C and Stringam GR (1997) Genus *Eruca*: a potential source of white rust resistance in cultivated brassicas. *Plant Genetic Resources Newsletter* 109: 25–26.

Barillari J, Canistro D, Paolini M, Ferroni F, Pedulli GF, Iori R and Valgimigli L (2005) Direct antioxidant activity of purified glucoerucin, the dietary secondary metabolite contained in rocket (*Eruca sativa* Mill.) seeds and sprouts. *Journal of Agricultural and Food Chemistry* 53: 2475–2482.

Bennett RN, Mellon FA, Botting NP, Eagles J, Rosa EAS and Williamson G (2002) Identification of the major glucosinolate (4-mercaptobutyl glucosinolate) in leaves of *Eruca sativa* L. (salad rocket). *Phytochemistry* 61: 25–30.

Burton WA, Ripley VL, Potts DA and Salisbury PA (2004) Assessment of genetic diversity in selected breeding lines and cultivars of canola quality *Brassica juncea* and their implications for canola breeding. *Euphytica* 136: 181–192.

Charcosset A and Moreau L (2004) Use of molecular markers for the development of new cultivars and the evaluation of genetic diversity. *Euphytica* 137: 81–94.

Curto G, Dallavalle E and Lazzeri L (2005) Life cycle duration of *Meloidogyne incognita* and host status of Brassicaceae and Capparaceae selected for glucosinolate content. *Nematology* 7: 203–212.

Das S, Tyagi AK and Singhal KK (2001) Chemical composition including amino acid, fatty acid and glucosinolate profile of taramira (*Eruca sativa*) oilseed. *Indian Journal of Agricultural Sciences* 71: 613–615.

Das S, Tyagi AK and Kaur H (2003) Evaluation of taramira oil-cake and reduction of its glucosinolate content by different treatments. *Indian Journal of Animal Sciences* 73: 687–691.

Excoffier L, Laval G and Schneider S (2006) *Arlequin ver 3.01. An integrated software package for population genetics data analysis*. University of Berne, Switzerland: Computational and Molecular Population Genetics Laboratory.

Fagbenro OA (2004) Soybean meal replacement by roquette (*Eruca sativa* Miller) seed meal as protein feedstuff in diets for African Catfish, *Clarias gariepinus* (Burchell 1822), fingerlings. *Aquaculture Research* 35: 917–923.

Gómez-Campo C (1993) *Eruca*. In: Castroviejo S, Aedo C, Gómez-Campo C, Laínz M, Montserrat P, Morales R, Muñoz Garmendia F, Nieto Feliner G, Rico E, Talavera S and Villar L (eds) *Flora Iberica. Vol IV. Cruciferae–Mono-*

tropaceae. Madrid: Real Jardín Botánico, CSIC, pp. 390–392.
Gómez-Campo C (1999) Taxonomy. In: Gómez-Campo C (ed.) *Biology of Brassica Coenospecies*. Amsterdam, The Netherlands: Elsevier Science B.V., pp. 3–32.
Gómez-Campo C (2003) Morphological characterisation of *Eruca vesicaria* (*Cruciferae*) germplasm. *Bocconea* 16: 615–624.
Gómez-Campo C and Prakash S (1999) Origin and domestication. In: Gómez-Campo C (ed.) *Biology of Brassica Coenospecies*. Amsterdam, The Netherlands: Elsevier Science B.V., pp. 33–58.
Greuter W, Burdet HM and Long G (eds) (1986) Cruciferae. *Med-checklist*. Optima, Geneva: Conservatoire et Jardin Botaniques de la Ville de Genève, Vol. 3, pp. 34–172.
Gurgar RSS, Sharma MM and Singh AK (1999) Stability analysis for seed yield and oil content in taramira (*Eruca sativa* Mill.) genotypes. *Annals of Biology* 15: 197–199.
Huh MK and Ohnishi O (2002) Genetic diversity and genetic relationships of East Asian natural populations of wild radish revealed by AFLP. *Breeding Science* 52: 79–88.
Jalas J, Suominen J and Lampinen R (eds) (1996) *Atlas Florae Europaeae – Distribution of Vascular Plants in Europe*. Vol. 11. Cruciferae (*Ricotia* to *Raphanus*). Helsinki, Finland: Helsinki University Printing House.
Li H, Barbetti MJ and Sivasithamparam K (2005) Hazard from reliance on cruciferous hosts as sources of major gene-based resistance for managing blackleg (*Leptosphaeria maculans*) disease. *Field Crops Research* 91: 185–198.
Lombard V, Baril CP, Dubreuil P, Blouet F and Zhang D (2000) Genetic relationships and fingerprinting of rapeseed cultivars by AFLP: consequences for varietal registration. *Crop Science* 40: 1417–1425.
Lühs W and Friedt W (1994) The major oil crops. In: Murphy DJ (ed.) *Designer Oil Crops: Breeding, Processing and Biotechnology*. Weinheim, Germany: VCH Verlagsgesellschaft, pp. 5–71.
Maire R (1965) *Flore de l'Afrique du Nord*. Vol. XII. Paris: Lechevalier.
Mandal S, Yadav S, Singh R, Begum G, Suneja P and Singh M (2002) Correlation studies on oil content and fatty acid profile of some Cruciferous species. *Genetic Resources and Crop Evolution* 49: 551–556.
Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research* 27: 209–220.
Matsuzawa Y, Mekiyanon S, Kaneko Y, Bang SW, Wakui K and Takahata Y (1999) Male sterility in alloplasmic *Brassica rapa* L. carrying *Eruca sativa* cytoplasm. *Plant Breeding* 118: 82–84.
Mohiuddin S, Qureshi RA, Qureshi SA, Nasir MKA and Khatri LM (1990) Studies on the repellent activity of some indigenous plant oils against *Tribolium castaneum* (Herbst.). *Pakistan Journal of Scientific and Industrial Research* 33: 326–328.
Muminović J, Merz A, Melchinger AE and Lübberstedt T (2005) Genetic structure and diversity among radish varieties as inferred from AFLP and ISSR analyses. *Journal of the American Society for Horticultural Science* 130: 79–87.
Negi MS, Sabharwal V., Bhat SR and Lakshmikumaran M (2004) Utility of AFLP markers for the assessment of genetic diversity within *Brassica nigra* germplasm. *Plant Breeding* 123: 13–16.
Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S and Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding* 2: 225–238.
Rana JS, Khokhar KS and Singh H (1995) Relative susceptibility of *Brassica* species to mustard aphid, *Lipaphis erysimi* (Kalt.). *Journal of Insect Science* 8: 96–97.
Rohlf FJ (1997) *NTSYSpc. Numerical taxonomy and multivariate analysis system version 2.02*. Setauket, New York: Exeter Software.
Rollins RC (1993) *The Cruciferae of Continental North America*. Stanford, California: Stanford University Press.
Rosa EAS (1999) Chemical composition. In: Gómez-Campo C (ed.) *Biology of Brassica Coenospecies*. Amsterdam, The Netherlands: Elsevier Science B.V., pp. 315–357.
Singh MP and Kolte SJ (1999) Differential reactions of various crucifer host species against isolates of *Peronospora parasitica*. *Journal of Mycology and Plant Pathology* 29: 118–121.
Singh R, Ellis PR, Pink DAC and Phelps K (1994) An investigation of the resistance to cabbage aphid in brassica species. *Annals of Applied Biology* 125: 457–465.
Singh SP and Rajput OP (1993a) Seed quality and oil yield of rocket-salad (*Eruca sativa*) as influenced by date of sowing and row spacing. *Indian Journal of Agronomy* 38: 335–336.
Singh SP and Rajput OP (1993b) Yield of rocket-salad (*Eruca sativa*) as affected by date of seeding and row spacing. *Indian Journal of Agronomy* 38: 603–605.
Sobotka R, Dolanská L, Čurn V and Ovesná J (2004) Fluorescence-based AFLPs occur as the most suitable marker system for oilseed rape cultivar identification. *Journal of Applied Genetics* 45: 161–173.
Sodani SN, Sastry EVD and Nehra MR (1990) Divergence analysis in taramira (*Eruca sativa* Mill.). *Indian Journal of Genetics and Plant Breeding* 50: 9–12.
Sosulski FW and Dabrowski KJ (1984) Determination of glucosinolates in canola meal and protein products by desulfation and capillary gas-liquid chromatography. *Journal of Agricultural and Food Chemistry* 32: 1172–1175.
Specht CE and Diederichsen A (2001) Eruca. In: Hanelt P (ed.) *Mansfeld's Encyclopedia of Agricultural and Horticultural Crops*. Vol. 3. Berlin, Germany: Springer-Verlag, pp. 1470–1472.
Srivastava A, Gupta V, Pental D and Pradhan AK (2001) AFLP-based genetic diversity assessment amongst agronomically important natural and some newly synthesized lines of *Brassica juncea*. *Theoretical and Applied Genetics* 102: 193–199.
Sun W, Pan Q, Liu Z, Meng Y, Zhang T, Wang H and Zeng X (2004) Genetic resources of oilseed *Brassica* and related species in Gansu Province, China. *Plant Genetic Resources: Characterization and Utilization* 2: 167–173.
Sun W, Pan Q, Liu Z, Meng Y, Zhang T, Wang H and Zeng X (2005) Overcoming self-incompatibility in *Eruca sativa* by chemical treatment of stigmas. *Plant Genetic Resources: Characterization and Utilization* 3: 13–18.
Tewari JP and Conn KL (1993) Reactions of some wild crucifers to *Alternaria brassicae*. *International Organisation for Biological and Integrated Control/uprs Bulletin* 16: 53–58.
Tewari JP, Bansal VK, Tewari I, Gómez-Campo C, Stringam GR and Thiagarajah MR (1996) Reactions of some wild and cultivated accessions of *Eruca* against *Leptosphaeria maculans*. *Eucarpia Cruciferae Newsletter* 18: 130–131.
Thies W (1971) Schnelle und einfache Analysen der Fettsäurezusammensetzung in einzelnen Raps-Kotyledonen I. Gaschromatographische und papierchromatographische Methoden. *Zeitschrift für Pflanzenzüchtung* 65: 181–202.
Tiyagi SA and Alam MM (1995) Efficacy of oil-seed cakes against plant-parasitic nematodes and soil-inhabiting fungi on mungbean and chickpea. *Bioresource Technology* 51: 233–239.

Tutin TG (1993) Eruca. In: Tutin TG, Burges NA, Chater AO, Edmondson JR, Heywood VH, Moore DM, Valentine DH, Walters SM and Webb DA (eds) *Flora Europaea*. Vol. 1, 2nd edn. Cambridge, UK: Cambridge University Press, p. 410.

Verma SC, Malik R and Dhir I (1977) Genetics of the incompatibility system in the crucifer *Eruca sativa* L. *Proceedings of the Royal Society of London (Series B)* 196: 131–159.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M and Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407–4414.

Warwick SI and Gugel RK (2003) Genetic variation in the *Crambe abyssinica–C. hispanica–C. glabrata* complex. *Genetic Resources and Crop Evolution* 50: 291–305.

Warwick SI, Gugel RK, McDonald T and Falk KC (2006) Genetic variation of Ethiopian mustard (*Brassica carinata* A. Braun) germplasm in western Canada. *Genetic Resources and Crop Evolution* 53: 297–312.

Yadava TP, Friedt DW and Gupta SK (1998) Oil content and fatty acid composition of taramira (*Eruca sativa* L.) genotypes. *Journal of Food Science and Technology* 35: 557–558.

Yaniv Z, Elber Y, Zur M and Schafferman D (1991) Differences in fatty acid composition of oils of wild cruciferae seed. *Phytochemistry* 30: 841–843.

Yaniv Z, Elber Y, Schafferman D, Ben-Moshe E and Zur M (1995) A survey of crucifers native to Israel, as a source of oils. *Plant Genetic Resources Newsletter* 101: 1–5.

Yaniv Z, Schafferman D and Amar Z (1998) Tradition, uses and biodiversity of rocket (*Eruca sativa*, Brassicaceae) in Israel. *Economic Botany* 52: 394–400.

Zhao J, Wang X, Deng B, Lou P, Wu J, Sun R, Xu Z, Vromans J, Koornneef M and Bonnema G (2005) Genetic relationships within *Brassica rapa* as inferred from AFLP fingerprints. *Theoretical and Applied Genetics* 110: 1301–1314.