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Genetic diversity of *Sinapis alba* germplasm as revealed by AFLP markers

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Abstract

Sinapis alba L. is a major specialty crop grown as a condiment in western Canada, but little is known about its genetic diversity. The objective of this study was to assess the level and pattern of genetic diversity in a collection of 127 *S. alba* accessions held at Plant Gene Resources of Canada using amplified fragment length polymorphism (AFLP) markers. Five AFLP primer pairs were applied, and 134 polymorphic bands were scored for each accession. These scored bands had frequencies of occurrence ranging from 0.02 to 0.99 with an average of 0.69. More AFLP variation was found within single (79.1%) than between (20.9%) *S. alba* accessions. A small degree of AFLP difference (1.7%) was observed among the accessions of various regions, while relatively large variation (9.2%) existed among the accessions of various countries. A large AFLP difference (15.6%) also existed between the yellow- and brown-seeded accessions, but only 6.2% difference was observed between the cultivar and landrace accessions. Two distinct groups of *S. alba* germplasm were identified on the basis of the seed colour (yellow or brown), although a few mixtures also existed. No apparent 'duplicated' accessions were observed. The most diverse accessions were from Italy, Spain, France and Greece. Among the most genetically distinct accessions were SA97 from Portugal, SA89 and SA88 from France, SA83 from Russia and SA57 from Italy. These findings are significant not only for managing *S. alba* germplasm, but also for identifying diverse germplasm that can be used by plant breeders to improve *S. alba* seed yield and quality parameters.

Keywords: AFLP; genetic diversity; genetic relationship; germplasm collection; *Sinapis alba*

Introduction

Sinapis alba L. (yellow mustard) is a major specialty crop that has been grown as a condiment in the western Canadian prairies since the 1940s (Downey and Rakow, 1995). Breeding began in the 1950s to increase seed yield, and since the 1970s additional breeding effort has been directed toward developing this species as an edible oilseed and high-protein crop for western Canada (Downey and Rakow, 1995; Katepa-Mupondwa *et al*., 2004). Low erucic acid (C22:1), low glucosinolate lines of *S. alba* have been developed at the Saskatoon Research Centre of Agriculture and Agri-Food Canada (Raney *et al*., 1995). To enhance these breeding programmes, efforts were also made over many years to collect *S. alba* germplasm from different parts of the world. Currently, a world collection of 127 *S. alba* accessions is maintained at Plant Gene Resources of Canada (PGRC) at Saskatoon. However, no molecular characterization of these accessions has been made, and little is known about the genetic variability of this collection.

Efforts have been made in some countries to assess the potential of *S. alba* as an oilseed crop (Olsson, 1974; Yaniv *et al*., 1994; Raney *et al*., 1995; Katepa-Mupondwa *et al*., 2004), but little attention has been paid to characterizing the genetic variability of this species using molecular techniques (Granot *et al*., 1996). Using random

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amplified polymorphic DNA (RAPD) markers (Williams *et al.*, 1990), Granot *et al.* (1996) revealed relatively large RAPD variations within and among *S. alba* accessions comparable to other outcrossing plants such as buffalograss (*Buchloe dactyloides*) (Huff *et al.*, 1993). However, this study was limited to only a few accessions and thus provided limited resolution on the genetic variability of this species. The amplified fragment length polymorphism (AFLP) technique, first described by Vos *et al.* (1995) as a tool for DNA fingerprinting, is technically more reproducible and cost-effective than RAPD markers. The AFLP technique has been widely applied to assess genetic variation in *Brassica* crop species including *B. napus* (Lombard *et al.*, 2000), *B. juncea* (Srivastava *et al.*, 2001) and *B. rapa* (Huh and Huh, 2001), but no studies on *S. alba* were found in the literature.

The objective of this study was to assess the level and pattern of genetic diversity in *S. alba* germplasm held in the PGRC collection using AFLP markers. It is our hope that this assessment will generate some baseline information not only for managing our *S. alba* germplasm collection, but also for identifying diverse germplasm for the improvement of *S. alba* seed yield and quality parameters.

Materials and methods

Plant materials

The PGRC *S. alba* germplasm was collected over several decades from 23 countries representing mainly Europe, Asia and North America (Table 1). The collection consists of cultivars, breeding lines, landraces and wild accessions. There are 95 yellow-seeded, 28 brown-seeded and four mixed seed colour accessions. Twelve seeds were randomly selected from each accession and grown in the greenhouse at the Saskatoon Research Centre. Young leaves were individually collected from 13-day-old seedlings, freeze-dried with a Labconco Freeze Dry System for 3–5 days, and stored at −80°C. Five accessions with 10 or more plants were randomly selected for individual plant assays to assess within-accession variation. For each accession, a bulk sample was also made with approximately equal amounts of dry leaves from each of the 8–12 plants to assess the genetic variation and relationships among accessions.

DNA isolation and AFLP analysis

Genomic DNA was extracted from single plant and bulk samples using the DNeasy Plant Mini Kit (Qiagen Inc., Mississauga, ON, Canada) according to the manufacturer's directions. Extracted DNA was quantified by fluorimetry using Hoechst 33 258 stain (Sigma Chemical Co., St Louis, MO, USA), then diluted to 25 ng/μl for AFLP analysis. The AFLP™ Analysis System 1 (Life Technologies, Burlington, ON, Canada) was applied following the protocol described by Vos *et al.* (1995) with exception of labelling with γ^{33}P.

Thirteen *Eco*RI:*Mse*I primer pairs were screened on 10 randomly selected bulk samples to assess the ability of these primer pairs to detect molecular variation. The five most informative pairs (Table 2) were selected for further AFLP analysis. To minimize technique-born and scoring errors, three bulk samples that appeared in the first gel were replicated in the second gel, and comparisons of the band patterns of replicated samples between gels were made to assess the consistency of scored AFLP bands.

Data analysis

The numbers of monomorphic and polymorphic AFLP bands were counted for each primer pair. Selective polymorphic AFLP bands with clarity for all the samples were manually scored as 1 (present) or 0 (absent), and these scored bands were assessed for inconsistency between replicates before being selected for further analysis. The selected polymorphic bands were analysed for the level of polymorphism by counting the number of polymorphic bands and generating summary statistics on the band frequencies. To visualize the pattern of variation, the numbers of polymorphic bands were plotted with respect to their frequencies of occurrence in all the assayed accessions.

For each accession represented by a bulk sample, the proportion of fixed recessive AFLP bands was calculated as the number of bands absent divided by the total number of selected bands. The absence of an AFLP band in an individual sample was assumed to be a recessive homozygote. With a bulked sample of 8–12 individuals per accession, the absence of an AFLP band at a locus also means a fixation of the recessive allele at a frequency greater than 0.9968 [(= $\sqrt[0.95]{2n}$ where n is the number of individuals bulked); Fu, 2000]. A higher proportion of fixed recessive bands obtained would mean lower genetic variation for an accession when compared with other accessions (Fu *et al.*, 2002). This relative measure allows comparisons of AFLP variations among the accessions of various groups by evaluating the means and variances of the proportion of fixed recessive AFLP bands. To assess the genetic uniqueness of an accession, average dissimilarity for each accession was calculated using the simple matching coefficient (Sokal and Michener, 1958) from all the possible pairs of the accession with the other accessions assayed. The higher

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Table 1. Characteristics of 127 *Sinapis alba* accessions used in the present study

Code[a]	CN[b]	Origin[c]	Type[d]	PFL[e]	DIST[f]
SA1	33 057	CAN(1)	Y/C	0.433	0.299
SA2	39 042	CAN(1)	Y/C	0.224	0.247
SA3	102131	GBR(4)	Y/C	0.410	0.295
SA4	43 807	GBR(4)	Y/C	0.291	0.241
SA5*	45 727	GBR(4)	Y/C	0.276	0.266
SA6*	10 2132	RUS(3)	Y/L	0.216	0.237
SA7	10 2133	FRA(4)	Y/C	0.321	0.269
SA8	10 2134	FRA(4)	Y/C	0.201	0.246
SA9	10 2135	UNK(5)	Y/L	0.463	0.303
SA10	10 2136	SWE(4)	Y/C	0.291	0.232
SA11	10 2137	SWE(4)	Y/C	0.343	0.256
SA12	10 2138	SWE(4)	Y/L	0.440	0.289
SA13	10 2139	SWE(4)	Y/L	0.425	0.286
SA14	10 2140	HUN(3)	Y/C	0.328	0.282
SA15	10 2141	HUN(3)	Y/C	0.299	0.248
SA16	10 2142	POL(3)	Y/C	0.224	0.223
SA17	10 2143	SWE(4)	Y/C	0.358	0.302
SA18	10 2144	CAN(1)	Y/C	0.179	0.249
SA19	10 2145	CAN(1)	Y/C	0.261	0.254
SA20	10 2146	DEU(4)	Y/C	0.366	0.273
SA21	10 2147	UNK(5)	Y/C	0.261	0.242
SA22	10 2148	DEU(4)	Y/C	0.306	0.249
SA23	10 2149	DEU(4)	Y/C	0.231	0.246
SA24	10 2150	UNK(5)	Y/C	0.246	0.228
SA25	10 2151	NLD(4)	Y/C	0.299	0.282
SA26	10 2152	UNK(5)	Y/C	0.351	0.252
SA27	10 2153	GBR(4)	Y/C	0.306	0.258
SA28	10 2154	DEU(4)	Y/C	0.299	0.257
SA29	30 473	RUS(3)	Y/L	0.231	0.232
SA30	10 2155	RUS(3)	Y/L	0.321	0.249
SA31*	10 2156	RUS(3)	Y/L	0.201	0.225
SA32	10 2157	UNK(5)	Y/C	0.306	0.259
SA33	10 2158	DEU(4)	Y/C	0.276	0.266
SA34	10 2159	UNK(5)	Y/C	0.299	0.246
SA35	10 2160	CZE(3)	Y/C	0.254	0.236
SA36	10 2161	UNK(5)	Y/C	0.261	0.247
SA37	10 2162	DEU(4)	Y/C	0.321	0.265
SA38	43 560	DEU(4)	Y/C	0.209	0.228
SA39	40 230	RUS(3)	Y/L	0.261	0.249
SA40	10 2163	UNK(5)	Y/C	0.351	0.290
SA41	10 2164	CZE(3)	Y/C	0.343	0.278
SA42	10 2165	NLD(4)	Y/C	0.321	0.264
SA43	10 2166	UNK(5)	Y/C	0.306	0.274
SA44	10 2167	UNK(5)	Y/C	0.321	0.263
SA45	10 2168	UNK(5)	Y/C	0.299	0.272
SA46*	10 2169	UNK(5)	Y/C	0.261	0.243
SA47	10 2170	NLD(4)	Y/C	0.299	0.253
SA48	10 2171	UNK(5)	Y/C	0.433	0.283
SA49	10 2172	UNK(5)	Y/C	0.284	0.251
SA50	10 2173	DEU(4)	Y/C	0.336	0.262
SA51	10 2174	UNK(5)	Y/C	0.254	0.222
SA52	10 2175	UNK(5)	Y/C	0.269	0.245
SA53	10 2176	DEU(4)	B/L	0.321	0.258
SA54	10 2177	DEU(4)	B/L	0.336	0.273
SA55	33 056	CAN(1)	Y/C	0.261	0.238
SA56	10 2178	UNK(5)	B/L	0.448	0.422
SA57	10 2179	ITA(4)	B/L	0.500	0.403
SA58	10 2180	ESP(4)	B/L	0.425	0.387
SA59	10 2181	ESP(4)	B/L	0.321	0.305
SA60*	10 2182	LVA(3)	Y/L	0.433	0.308

Table 1. *Continued*

Code[a]	CN[b]	Origin[c]	Type[d]	PFL[e]	DIST[f]
SA61	10 2183	DEU(4)	Y/L	0.269	0.235
SA62	10 2184	DEU(4)	Y/L	0.313	0.246
SA63	10 2185	ISR(2)	BY/L	0.313	0.247
SA64	10 2186	CAN(1)	Y/C	0.254	0.224
SA65	10 2187	DEU(4)	Y/L	0.231	0.239
SA66	10 2188	RUS(3)	Y/L	0.231	0.232
SA67	10 2189	RUS(3)	Y/L	0.254	0.252
SA68	10 2190	LTU(3)	Y/L	0.254	0.241
SA69	10 2191	RUS(3)	Y/L	0.313	0.230
SA70	10 2192	RUS(3)	Y/L	0.254	0.226
SA71	10 2193	MNG(2)	Y/L	0.328	0.263
SA72	10 2194	RUS(3)	BY/L	0.336	0.262
SA73	10 2195	UKR(3)	Y/L	0.254	0.234
SA74	10 2196	BGR(3)	Y/L	0.269	0.249
SA75	10 2197	RUS(3)	Y/L	0.231	0.245
SA76	10 2198	GRC(3)	Y/L	0.216	0.238
SA77	10 2199	GRC(3)	Y/L	0.299	0.268
SA78	10 2200	ITA(4)	Y/L	0.328	0.246
SA79	10 2201	GRC(3)	Y/L	0.246	0.259
SA80	10 2202	PRK(2)	Y/L	0.269	0.238
SA81	10 2203	PRK(2)	Y/L	0.276	0.254
SA82	10 2204	PRK(2)	Y/L	0.284	0.255
SA83	10 2205	RUS(3)	Y/L	0.627	0.417
SA84	10 2206	ITA(4)	Y/L	0.366	0.275
SA85	10 2207	LTU(3)	Y/L	0.358	0.253
SA86	10 2208	CAN(1)	Y/L	0.366	0.282
SA87	10 2209	ROM(3)	Y/L	0.373	0.275
SA88	10 2210	FRA(4)	B/L	0.470	0.373
SA89	10 2211	FRA(4)	B/L	0.440	0.330
SA90	10 2212	ITA(4)	BY/L	0.358	0.299
SA91	10 2213	ITA(4)	BY/L	0.313	0.226
SA92	10 2214	FRA(4)	B/L	0.403	0.327
SA93	10 2215	ITA(4)	B/L	0.351	0.359
SA94	10 2216	ISR(2)	B/L	0.291	0.314
SA95	10 2217	ITA(4)	B/L	0.313	0.317
SA96	10 2218	ESP(4)	B/L	0.299	0.301
SA97	10 2219	PRT(4)	B/L	0.649	0.473
SA98	10 2220	ITA(4)	B/L	0.440	0.327
SA99	10 2221	GRC(3)	B/L	0.187	0.290
SA100	10 2222	ITA(4)	B/L	0.366	0.354
SA101	10 2223	GRC(3)	B/L	0.321	0.329
SA102	10 2224	ISR(2)	B/L	0.239	0.303
SA103	10 2225	ISR(2)	B/L	0.291	0.284
SA104	10 2226	ISR(2)	B/L	0.261	0.325
SA105	10 2227	ISR(2)	B/L	0.313	0.307
SA106	43 449	ESP(4)	Y/L	0.403	0.293
SA107	43 450	UNK(5)	Y/L	0.366	0.294
SA108	45 814	CAN(1)	Y/L	0.194	0.239
SA109	91 065	UNK(5)	Y/C	0.321	0.279
SA110	10 7302	CAN(1)	Y/C	0.246	0.238
SA111	10 7303	ITA(4)	B/L	0.351	0.386
SA112	10 7304	ITA(4)	B/L	0.448	0.353
SA113	10 7305	GRC(3)	B/L	0.381	0.378
SA114	10 7306	GRC(3)	B/L	0.403	0.380
SA115	10 7307	GRC(3)	B/L	0.425	0.340
SA116	10 7308	GRC(3)	B/L	0.336	0.309
SA117	10 7309	AUT(4)	Y/C	0.291	0.261
SA118	10 7310	UNK(5)	Y/C	0.187	0.239
SA119	10 7311	ROM(3)	Y/C	0.224	0.244
SA120	10 7312	HUN(3)	Y/C	0.231	0.236
SA121	10 7313	DEU(4)	Y/C	0.239	0.240

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Table 1. *Continued*

Code[a]	CN[b]	Origin[c]	Type[d]	PFL[e]	DIST[f]
SA122	107314	DEU(4)	Y/C	0.261	0.237
SA123	107315	DEU(4)	Y/C	0.261	0.240
SA124	107316	FRA(4)	Y/C	0.224	0.249
SA125	107317	DEU(4)	Y/C	0.239	0.229
SA126	107318	CZE(3)	Y/C	0.269	0.236
SA127	107319	DEU(4)	Y/C	0.246	0.253

[a] Accessions with * were also assessed for within-accession variation.
[b] Canadian National accession number in the PGRC collection.
[c] Country (and region) of origin. Country code follows the ISO code except for UNK, which means country of origin is unknown or uncertain. Region code in parentheses consists of 1 (North America), 2 (Asia), 3 (East Europe), 4 (West Europe) and 5 (other regions and/or unknown).
[d] Yellow (Y) and/or brown (B) seed colour of an accession known to be either a breeding line/cultivar (C) or a wild collection/landrace (L).
[e] Proportion of fixed recessive AFLP loci.
[f] Average dissimilarity of an accession compared with the rest of the accessions.

the average dissimilarity an accession has, the larger the genetic difference there is between this accession and the remaining 126 accessions.

To assess within-accession variation, analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) was performed (Arlequin version 2.001; Schneider *et al.*, 2002) on 50 individual plants of the five accessions selected for this purpose. To assess the variation among accessions of various groups (region, country, seed colour, landrace), AMOVAs were performed on all 127 bulk samples. These analyses also generated the average pairwise difference among the accessions within a group for the comparison of within-group variation. The significance of the resulting variance components was tested with 10,100 random permutations.

To assess the genetic relationships of individual accessions, a pairwise accession similarity matrix for 127 accessions was generated using the simple matching coefficient and clustered using NTSYS-PC 2.01 with the algorithm of unweighted pair-group methods using arithmetic averages (Rohlf, 1997). The similarity matrix was also converted to the Euclidean distance matrix for a principal coordinate analysis (NTSYS-pc). The first three resulting principal coordinate scores were plotted to determine the accession association.

Results and discussion

Five AFLP primer pairs amplified a total of 337 AFLP bands for the 127 *S. alba* accessions assayed from bulk sampling (Table 2). The number of observable bands per primer pair ranged from 41 to 108 and the number of monomorphic bands per primer pair ranged from 1 to 7. Such a high level of polymorphism is expected for an outcrossing species like *S. alba* (Olsson, 1960) and is comparable with those reported for some *Brassica* crop species (Lombard *et al.*, 2000; Huh and Huh, 2001; Srivastava *et al.*, 2001). Since many of the detected bands lacked clarity, only a proportion of the polymorphic bands were scored; 134 of the scored bands were selected for further analysis. These selected bands were presumably detected randomly from the $2n$ (=24) *S. alba* chromosomes and would have covered the whole genome, but the exact extent of genome coverage by these AFLP loci is not known. The frequencies of the selected bands in the assayed accessions ranged from 0.02 to 0.99 with an average of 0.69. A large proportion (45%) of the selected bands had frequencies larger than 0.85 (results not shown). For each primer pair, statistics (mean, minimum, maximum) of the band frequencies are given in Table 2; the mean frequencies for these primer pairs ranged from 0.65 to 0.72. Thus, a large amount of genetic diversity existed in these *S. alba* accessions.

Genetic variation within accessions

Screening individual plants from an accession allows inferences to be made on within-accession variation. This was done with five yellow-seeded accessions (SA5, SA6, SA31, SA46, SA60) that originated from at least three different

Table 2. AFLP variations revealed by five AFLP primer pairs in 127 *Sinapis alba* accessions

	Number of AFLP bands[a]			Frequency of scored bands		
Primer pair	Total	Mono	Scored	Mean	Minimum	Maximum
E + AAC/M + CAT	108	7	40	0.690	0.031	0.992
E + AAC/M + CTC	52	3	34	0.697	0.110	0.992
E + AAG/M + CAT	91	7	21	0.717	0.173	0.992
E + ACC/M + CTC	45	4	10	0.703	0.417	0.992
E + ACT/M + CTT	41	1	29	0.647	0.023	0.992
All	337	22	134	0.691	0.023	0.992

[a] Total, total number of AFLP bands observed; Mono, number of monomorphic AFLP bands detected; Scored, number of polymorphic AFLP bands scored.

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countries; two of these (SA5, SA46) were cultivars. The same primer pairs used for bulked samples (Table 2) were applied and a total of 122 polymorphic bands specific to these individual samples were selected for this analysis. These bands had frequencies ranging from 0.02 to 0.98 with an average of 0.53. Only two polymorphic bands of fragment sizes 279 and 352 bp that were amplified by the primer pair E + AAC/M + CTC were unique to accessions SA31 and SA46, respectively. Analysis of molecular variance revealed 79% of the total AFLP variance resided within the five accessions, indicating large genetic heterogeneity within each accession. This level of within-accession variation is expected for an outcrossing species (Srivastava *et al.*, 2001). The accession with the highest within-accession variation measured by the average pairwise difference was SA6 (38.4), followed by SA31 (37.1), SA5 (33.4), SA46 (32.8) and SA60 (23.5). These results, although only applicable to yellow-seeded accessions, suggest that the *S. alba* accessions held in the PGRC collection are genetically heterogeneous.

Genetic variation among accessions

Genetic variation for each accession was inferred from bulk sampling and measured by the proportion of fixed recessive AFLP loci and the average dissimilarity. The proportions of fixed recessive AFLP loci ranged from 0.179 to 0.649 with a mean of 0.312, and the average dissimilarities ranged from 0.222 to 0.473 with an average of 0.275 (Table 1). The accession with the highest proportion of fixed AFLP loci and potentially the lowest variation was the brown-seeded Portuguese accession SA97 (0.649), followed by the yellow-seeded Russian accession SA83 (0.637). The accession with the highest average dissimilarity and probably the most genetic uniqueness was also the Portuguese accession SA97 (0.473), followed by another brown-seeded accession of uncertain origin, SA56 (0.422). These results not only indicated the possible level of genetic variation, but also showed the genetic uniqueness for each accession assayed.

Variation among the 127 accessions was assessed based on four types of grouping (region, country, seed colour, landrace; Table 3). Within-group variation was measured not only by the mean of the proportions of fixed recessive AFLP loci and the average dissimilarities, but also by the average pairwise difference calculated from AMOVA for all the accessions within a group. The last measure should be most informative, as it takes into account both the number of polymorphic bands and their frequencies. For the four major regions, the West European accessions had the highest mean proportion of fixed recessive AFLP loci (0.334) and the highest dissimilarity average (0.285). This suggests that the accessions from the West Europe region generally have fewer polymorphic loci and are genetically more distinct than the accessions from the other regions. When both the number of polymorphic bands and their frequencies are taken into account as in AMOVA, these West European accessions still hold the highest within-group variation (39.3). In contrast, the North American accessions had the lowest mean proportion of fixed recessive AFLP loci (0.269) and the lowest average dissimilarity (0.252), which explains why the within-group variation for the accessions from this region was the lowest (29.5). Similar patterns were observed at the country level. The 11 Italian accessions had the highest mean proportion of fixed recessive AFLP loci (0.376) and the highest dissimilarity average (0.322), thus displaying the highest within-group variation (45.9). In contrast, the 18 German accessions had a relatively lower mean proportion of fixed recessive AFLP loci (0.281) and a lower dissimilarity average (0.250), which was reflected in a relatively low within-group variation (29.1). The most diverse accessions measured by the average pairwise difference among all the accessions within a country were from Italy (45.9), Spain (41.7), France (41.6) and Greece (40.9). The least diverse accessions were from the Netherlands (24.7), Korea (25.3) and Sweden (26.6). Clearly, the brown-seeded accessions had much more within-group variation (44.5) than the yellow-seeded accessions (31.0). Similarly, the landrace accessions had more within-group variation (40.6) than the cultivar accessions (29.9). However, when measured with the mean proportion of fixed recessive AFLP loci, brown-seeded or landrace accessions would have the lower within-group variation than yellow-seeded or cultivar accessions, respectively (Table 3). This discrepancy may reflect the bias in the inference of within-accession variation with the proportion of fixed recessive AFLP loci calculated from a bulk sample.

Partitioning of the total AFLP variation into within- and among-group components by AMOVA revealed significant differences among *S. alba* accessions of various groups (Table 4). More AFLP variation was found within single (79.1%) than among (20.9%) *S. alba* accessions. A relatively small difference (1.7%) was observed among the accessions of various regions, but relatively large variation (9.2%) existed among the accessions of various countries. A large AFLP difference (15.6%) also existed between the yellow- and brown-seeded accessions, but only 6.2% difference was observed between the cultivar and landrace accessions. These results suggest that seed colour can be used to differentiate among *S. alba* accessions and that brown-seeded accessions are genetically more heterogeneous than yellow-seeded accessions.

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Table 3. Comparison of AFLP variations for 127 *Sinapis alba* accessions grouped by region, country, seed colour and landrace

Group	Group size[a]	Mean PFL[b]	Mean DIST[c]	WG variation[d]
Region				
North America	9	0.269	0.252	29.5
Asia	10	0.287	0.279	35.2
East Europe	35	0.297	0.268	35.0
West Europe	53	0.334	0.285	39.3
Other	20	0.314	0.268	34.2
Country				
Canada	9	0.269	0.252	29.5
Korea	3	0.276	0.249	25.3
Israel	6	0.285	0.297	33.4
Former Czechoslovakia	3	0.289	0.250	28.7
Russia	12	0.290	0.255	31.0
Hungary	3	0.286	0.255	33.3
Greece	9	0.313	0.310	40.9
France	6	0.343	0.299	41.6
Germany	18	0.281	0.250	29.1
Italy	11	0.376	0.322	45.9
Netherlands	3	0.306	0.266	24.7
Spain	4	0.362	0.322	41.7
Sweden	5	0.371	0.273	26.6
UK	4	0.321	0.265	31.7
Unknown	20	0.314	0.268	34.2
Seed colour				
Yellow	95	0.295	0.256	31.0
Brown	32	0.364	0.329	44.5
Landrace				
Cultivar	59	0.286	0.254	29.9
Landrace	68	0.335	0.293	40.6

[a] Number of accessions within a group.
[b] Mean proportion of fixed AFLP loci for all the accessions within a group.
[c] Mean of the average dissimilarities for all the accessions within a group.
[d] Within-group variation calculated as the average pairwise difference among all the accessions within a group.

Genetic relationships among accessions

Similarities reflected in the 134 AFLP loci were calculated among all the accessions examined and clustered into two major groups at the similarity level of ≈0.71 (Fig. 1). The upper group included 99 mostly yellow-seeded accessions and the lower group 28 mostly brown-seeded accessions. In the large yellow-seeded group, there were two brown-seeded accessions (SA53, SA54) and three (SA63, SA72, SA91) that were mixtures of brown and yellow seed. In the brown-seeded group, there was only one yellow-seeded accession (SA83) and one accession with brown and yellow seeds (SA90). The most distinct accessions were SA97 from Portugal, SA89 and SA88 from France, SA83 from Russia and SA57 from Italy. Only one pair of accessions, SA40 and SA47, were likely duplicated, as they displayed a relatively high pairwise similarity (≈0.91).

A plot of the first two principal component scores based on the Euclidean distances converted from the simple matching coefficient matrix of 134 AFLP bands for the 127 *S. alba* accessions clearly revealed two groups (Fig. 2). When accessions were identified by their seed colours (yellow, brown, brown + yellow), two distinct groups were evident; these groups were essentially the yellow-seeded group (left side of Fig. 2A) and the brown-seeded group (right side of Fig. 2A) as described above. Excluding the outlier accession SA83 at the top, the yellow-seeded group was genetically more narrow than the brown-seeded group. The yellow-seeded group also included the four accessions of mixed seed colour.

It is not clear why seed colour clearly distinguishes among *S. alba* germplasm, although genetic shift induced from a long-term selection and breeding of *S. alba* could be a major factor, as only yellow-seeded *S. alba* germplasm was a breeding target over time. Granot *et al.* (1996) demonstrated a link between *S. alba* genotype and erucic acid content of the seed oil. Erucic acid contents were determined in a related study of 73 yellow-seeded and 22 brown-seeded accessions from the PGRC collection (R. Gugel and P. Raney, unpublished).

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Table 4. Analysis of molecular variance (AMOVA) sum of squares partitioning of total AFLP variation into among- and within-group components for 127 *Sinapis alba* accessions according to various models of structuring

Group[a]	df	Among-group component (%)[b]	Within-group component (%)
Accession	4	20.9****	79.1
Country	13	9.2****	90.8
Region	4	1.7**	98.3
Seed colour	1	15.6****	84.4
Landrace	1	6.2****	93.8

[a] The grouping was made to assess variations among and within five accessions (accession), 14 originating countries (country), five representative regions (region), 95 yellow and 32 brown accessions (seed colour), and 59 cultivated and 68 landrace accessions (landrace).
[b] The probability that the among-group (accession, country, region, seed colour, landrace) variance component was larger than zero, as computed from 10,100 random permutations. Significance: $^{**}P < 0.01$; $^{****}P < 0.0001$.

When the accessions were identified by their erucic acid contents, all 22 brown-seeded accessions had erucic acid contents above the value of the test mean (38.95%) and most of the yellow-seeded accessions had erucic acid contents below the value of the test mean (Fig. 2B). Further studies of oil profile and fatty acid composition may help explain the association of seed colour with *S. alba* genotype.

Implications for S. alba breeding and germplasm management

This study was conducted to characterize the extent, distribution and structure of genetic diversity in the PGRC collection of *S. alba* germplasm. It was found that large AFLP variation existed in the collection. More AFLP variation was found within single (79.1%) than among (20.9%) *S. alba* accessions. A small AFLP difference (1.7%) was observed among the accessions of various regions, while relatively large variation (9.2%) existed among the accessions of various countries. A large AFLP difference (15.6%) also existed between the yellow- and brown-seeded accessions, but only 6.2% difference was observed between the cultivar and landrace accessions. Two distinct groups of *S. alba* germplasm were identified by the seed colour (yellow or brown) of the accessions, although a few mixtures also existed. No apparent 'duplicated' accessions were observed. The most diverse accessions were from Italy, Spain, France and Greece. Among the most genetically distinct accessions were SA97 from Portugal, SA89 and SA88 from France, SA83 from Russia and SA57 from Italy. These findings are significant not only for managing *S. alba* germplasm collection, but also for identifying diverse germplasm that can be used by plant breeders to improve *S. alba* seed yield and quality parameters.

The findings presented here have several implications for *S. alba* germplasm management. First, the brown-seeded accessions displayed more genetic variability than the yellow-seeded accessions, implying that efforts should be directed toward acquiring and conserving more brown-seeded germplasm. Second, the landrace accessions harbour more variation than the cultivated materials. Thus, more emphasis should be given to the acquisition of germplasm collected directly from wild populations. Third, while the most diverse accessions were from the West Europe region, acquiring diverse germplasm still needs to be country-specific. Germplasm from the Netherlands, Sweden and Germany were usually less diverse than those from Italy, Spain and Greece. Fourth, although only one likely 'duplicated' accession was found, genetic redundancy exists particularly in the yellow-seeded group (Fig. 2).

This study demonstrated that the current breeding materials reflected in the yellow-seeded group has harboured less genetic diversity than those in the brown-seeded group representing many of the landrace or wild materials, which underscores the need for expanding the existing cultivated gene pool. A large amount of genetic diversity (90.8%) was found within *S. alba* accessions originating from certain countries such as Italy and Spain, implying that selection of breeding materials from accessions of certain countries for *S. alba* improvement is possible. More opportunity exists in the brown-seeded and landrace accessions to diversify the genetic basis, but removing the undesirable brown seed colour may pose a challenge for breeders. The genetic uniqueness described in this study (Table 1; Fig. 2) provides useful information for plant breeders to identify parental genotypes for hybrid combinations, particularly in the yellow-seeded group. Exploring new sources of germplasm for seed yield and quality parameters should be focused initially on accessions originating from the West Europe region, particularly from Italy, Spain and Greece, as germplasm from these countries appears to

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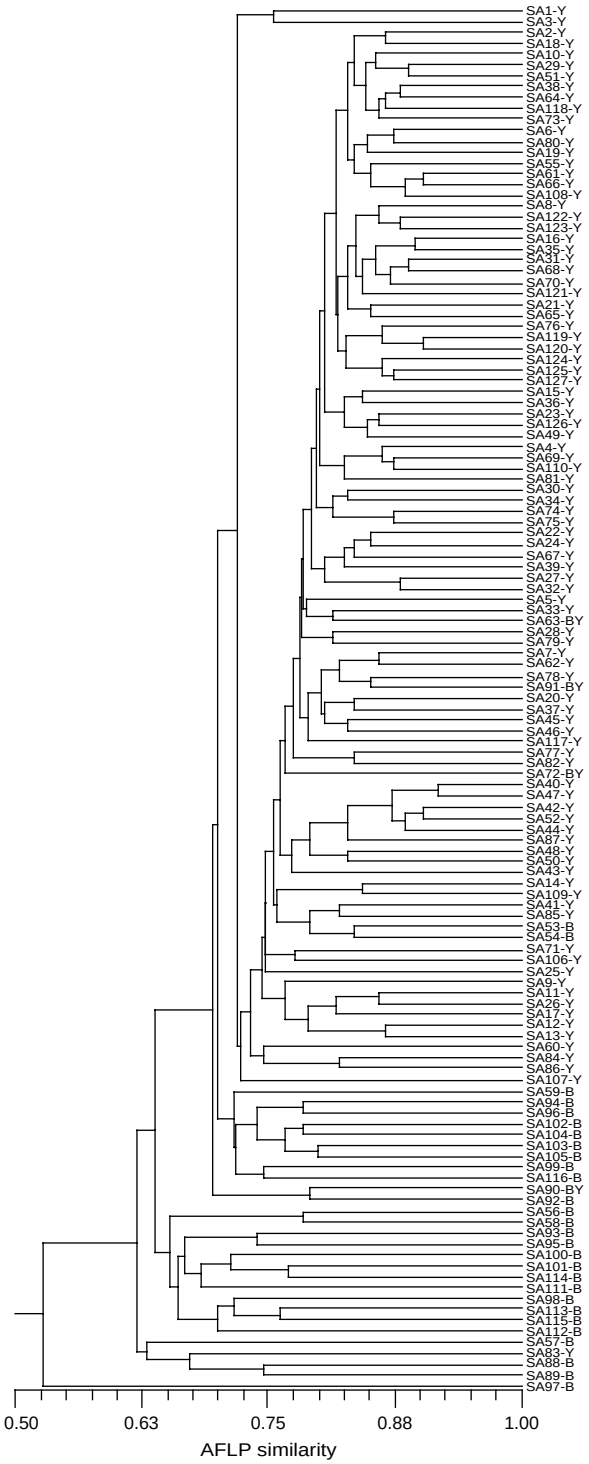

Fig. 1. Genetic relationships of 127 *Sinapis alba* accessions based on the similarity of 134 AFLP bands. Accession labels include the code and seed colour (Table 1).

be more diverse. As demonstrated, AFLP exhibited a high level of efficiency in detecting DNA polymorphism among *S. alba* accessions and thus could serve as a valuable tool for *S. alba* breeders.

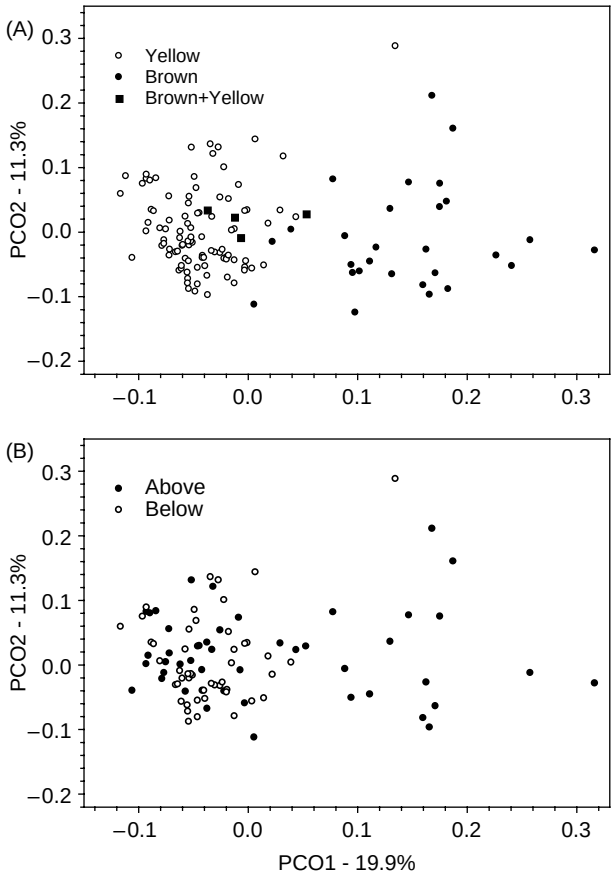

Fig. 2. Plot of the first two principal component scores based on the Euclidean distances converted from the simple matching coefficient matrix of 134 AFLP bands for 127 *Sinapis alba* accessions. These first two components accounted for 19.9% and 11.3% of the total variance, respectively. (A) Accessions (127) identified by their seed colours (yellow, brown or brown + yellow). (B) Accessions (95) labelled with the erucic acid content (% of total fatty acids) of their seed oils from a field test conducted in 1998; 'Above' or 'Below' refers to accessions with erucic acid contents above or below the value of the test mean (38.95%).

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