Genetic structure and diversity of a collection of *Brassica rapa* subsp. *rapa* L. revealed by simple sequence repeat markers

P. SOENGAS*, M. E. CARTEA, M. FRANCISCO, M. LEMA AND P. VELASCO

Misión Biológica de Galicia (CSIC), PO Box 28, E-36080-Pontevedra, Spain

(Revised MS received 7 December 2010; Accepted 13 December 2010; First published online 24 February 2011)

SUMMARY

Brassica rapa subsp. *rapa* L. includes three different crops: turnips (roots), turnip greens (leaves) and turnip tops (inflorescences). A collection of *B. rapa* subsp. *rapa* from north-western Spain is currently kept at 'Misión Biológica de Galicia' (a research centre of the Consejo Superior de Investigaciones Científicas (CSIC), Spain). This collection has been characterized based on morphological and agronomical traits. A better understanding of the genetic diversity present in the collection is necessary in order to optimize its use and maintenance. The objectives of the present work were to assess the genetic diversity present in the *B. rapa* subsp. *rapa* collection, to establish genetic relationships among populations and to study the genetic structure of the collection. Eighty populations were analysed based on 18 simple sequence repeats (SSRs). Populations showed a broad range of genetic diversity, thus offering good potential for further genetic improvement. Most of the variability was found within the population level, probably due to high rates of allogamy, to migration and/or interchange of seed among local growers. Populations showed a low level of differentiation, grouping in just one cluster, and therefore they can be considered as samples of a highly variable metapopulation that can be used for *B. rapa* breeding programmes.

INTRODUCTION

Brassica rapa L. is an obligate out-crossing crucifer grown worldwide as a vegetable and for edible and industrial oil, providing a large proportion of the daily food intake in many regions of the world (Ofori *et al.* 2008). There is a large variation in the plant organs that are consumed, which has resulted in the selection of different morphotypes depending on local preferences (Zhao *et al.* 2005). These crops are grown for their leaves (turnip leaves, Chinese cabbage, pak-choi, Narinosa, Komatsuna, Mizuna and Mibuna), seeds (turnip rape, Chinese turnip rape and Sarson types), inflorescences (Broccoletto, Caixin, turnip inflorescences and Zicaitai) and enlarged roots (turnips).

B. rapa is classified into different subspecies. *B. rapa* subsp. *rapa* L. includes plants used as three different crop types: turnips, turnip leaves and turnip inflorescences. It is believed that *B. rapa* subsp. *rapa* was first used for its nutritious root in Europe, which

occurred around 2500–2000 BCE (Gómez-Campo & Prakash 1999). The spread of vegetable crops within this subspecies, such as turnip inflorescences and turnip leaves, occurred later, independently from the origin of other leafy forms in Asia (Gómez-Campo & Prakash 1999). The long history and extensive use of cultivation of *Brassica* species in Galicia (north-western Spain) has given rise to a great number of landraces. They have been under cultivation for a very long time since they were among the first vegetables to be introduced into the Western Iberian Peninsula (Gómez-Campo & Prakash 1999).

B. rapa subsp. *rapa* L. is grown during the winter in the north-west of Spain, where turnip leaves and turnip inflorescences are used for human consumption in soups and stews, and turnips are mostly used for fodder. Agriculture in north-western Spain is traditional and even today growers continue to cultivate landraces in vegetable gardens for their own consumption. Commercial varieties of turnips, turnip leaves and turnip inflorescences have been selected by their use. Varieties employed as turnips have thickened roots, and they do not produce much leaf

* To whom all correspondence should be addressed.
Email: psoengas@mbg.cesga.es

Table 1. *List of Brassica rapa populations used in this study, including geographical origin, number of alleles and expected heterozygosity*

Accession name	Geographical origin (Galicia province)	Location	Number of alleles	Expected heterozygosity
MBG-BRS0026	Pontevedra	Paramos, Tui	44	0.494
MBG-BRS0036	Pontevedra	Redondela	42	0.387
MBG-BRS0050	Pontevedra	Mogor, Marín	48	0.470
MBG-BRS0066	Pontevedra	Barro	44	0.389
MBG-BRS0069	Pontevedra	Barro	45	0.438
MBG-BRS0075	Pontevedra	Lourido, Poio	50	0.442
MBG-BRS0081	Pontevedra	Forcarey	45	0.382
MBG-BRS0082	Pontevedra	Forcarey	41	0.369
MBG-BRS0089	Pontevedra	Carballida, Salceda de Caselas	42	0.327
MBG-BRS0099	A Coruña	Trazo, Ordes	35	0.289
MBG-BRS0101	Pontevedra	Cuntis	46	0.369
MBG-BRS0102	Pontevedra	La Estrada	54	0.482
MBG-BRS0116	Pontevedra	Vila de Cruces	48	0.411
MBG-BRS0123	Pontevedra	Lalín	33	0.265
MBG-BRS0124	Pontevedra	Lalín	42	0.424
MBG-BRS0125	Pontevedra	Lalín	51	0.496
MBG-BRS0132	Pontevedra	Salcedo, Pontevedra	48	0.410
MBG-BRS0139	A Coruña	Trobe, Vedra	55	0.527
MBG-BRS0140	A Coruña	Lama, Boqueixón	54	0.519
MBG-BRS0143	A Coruña	Lama, Boqueixón	50	0.489
MBG-BRS0154	A Coruña	Ordes	48	0.476
MBG-BRS0155	Ourense	Pereiro de Aguiar	54	0.495
MBG-BRS0159	Ourense	Lamas, Leiro	48	0.447
MBG-BRS0163	Ourense	Barcia, Melón	44	0.416
MBG-BRS0171	Ourense	San Cristóbal, Ribadavia	47	0.462
MBG-BRS0173	Ourense	Valongo, Cortegada	55	0.512
MBG-BRS0175	Ourense	Crespos, Padrenda	53	0.499
MBG-BRS0179	A Coruña	Betanzos	53	0.473
MBG-BRS0180	A Coruña	Betanzos	51	0.474
MBG-BRS0181	A Coruña	Betanzos	48	0.472
MBG-BRS0183	A Coruña	Carballo	52	0.500
MBG-BRS0184	A Coruña	Carballo	54	0.470
MBG-BRS0186	Ourense	A Xironda, Cualedro	54	0.523
MBG-BRS0188	Ourense	Soutoverde, A Bola	47	0.527
MBG-BRS0191	Ourense	Alonga, Toén	49	0.496
MBG-BRS0193	Ourense	Castrelo de Miño	48	0.525
MBG-BRS0194	Ourense	Barral, Castrelo de Miño	48	0.493
MBG-BRS0195	Ourense	Cartelle	47	0.489
MBG-BRS0202	Ourense	Meás, Lobeira	45	0.446
MBG-BRS0203	Ourense	Maus de Baños, Bande	48	0.481
MBG-BRS0206	Ourense	Bembibre, Viana do Bolo	48	0.487
MBG-BRS0215	Ourense	Cameija, Boborás	47	0.446
MBG-BRS0216	Ourense	Maside	45	0.511
MBG-BRS0220	Ourense	Amoeiro	47	0.493
MBG-BRS0222	Ourense	Vilar de San Ginés, A Peroxa	52	0.427
MBG-BRS0224	Ourense	Conchouso, A Peroxa	53	0.530
MBG-BRS0228	Ourense	Ordelles, Pereiro de Aguiar	45	0.455
MBG-BRS0232	Ourense	Tarreirigo, Esgos	46	0.476
MBG-BRS0233	Ourense	Chaloso, Maceda	49	0.495
MBG-BRS0234	Ourense	Chaloso, Maceda	48	0.445
MBG-BRS0235	Ourense	Chaloso, Maceda	49	0.518
MBG-BRS0236	Ourense	Chaloso, Maceda	45	0.472
MBG-BRS0237	Ourense	Sanguiniedo, Baños de Molgas	46	0.492
MBG-BRS0242	Ourense	Vilar de Flores, Allariz	52	0.518
MBG-BRS0244	Ourense	Barrio, Xunqueira de Espadanedo	52	0.482
MBG-BRS0254	Ourense	A Teixeira	55	0.487
MBG-BRS0256	Ourense	A Teixeira	49	0.420

Table 1. (Cont.)

Accession name	Geographical origin (Galicia province)	Location	Number of alleles	Expected heterozygosity
MBG-BRS0266	Ourense	Vozqueimados, Chandrexa de Queixa	54	0.485
MBG-BRS0274	Ourense	San Juan de Barrio, A Proba de Trives	53	0.511
MBG-BRS0282	Ourense	Matosiños, La Merca	51	0.456
MBG-BRS0284	Ourense	Meronzo Grande, La Merca	56	0.536
MBG-BRS0304	Ourense	Gomariz, Baltar	50	0.504
MBG-BRS0312	Ourense	Vilar de Barrio	47	0.390
MBG-BRS0322	Ourense	Flariz, Monterrei	57	0.487
MBG-BRS0401	A Coruña	San Xiao, Coirós	43	0.375
MBG-BRS0430	Lugo	Santaballa, Vilalba	51	0.472
MBG-BRS0433	A Coruña	Santiago	49	0.459
MBG-BRS0440	Lugo	Llacin, Carballido, A Fonsagrada	37	0.302
MBG-BRS0450	A Coruña	Lago, Valdoviño	40	0.436
MBG-BRS0451	A Coruña	O Val, Narón	51	0.475
MBG-BRS0459	Lugo	Alba, Palas de Rey	49	0.443
MBG-BRS0461	Lugo	Castro de Rei	41	0.338
MBG-BRS0471	Lugo	San Julián de Tor, Monforte de Lemos	44	0.413
MBG-BRS0472	A Coruña	Porta, Sobrado	48	0.447
MBG-BRS0496	A Coruña	Fosado, Montao, Ordes	39	0.421
MBG-BRS0544	A Coruña	O Castro, Armentón, Arteixo	45	0.433
MBG-BRS0547	Lugo	Abadín	44	0.450
MBG-BRS0550	A Coruña	Trazo	50	0.442
MBG-BRS0552	Portugal	Lapa-Moimenta	44	0.444
MBG-BRS0553	Portugal	Pias-Monçao	49	0.510
Mean			48	0.455

or secondary stems, and so they are not suitable for obtaining turnip leaves or turnip inflorescences. Commercial varieties grown to obtain turnip inflorescences and turnip leaves have been selected for high production of floral shoots or leaves. However, in the case of many local landraces grown in Galicia, the same landrace can be grown for more than one purpose, allowing the existence of local populations with high levels of variability (Padilla *et al.* 2005). For example, within some landraces there are plants that have thickened roots and others that produce high numbers of leaves.

These landraces are a valuable resource since they are highly variable, are adapted to the climatic conditions of the area and can be resistant to different pests and diseases. A collection of *B. rapa* subsp. *rapa* from north-western Spain is currently kept at 'Misión Biológica de Galicia' (CSIC, Spain). This collection has been characterized based on morphological and agronomical traits (Padilla *et al.* 2005); some landraces have been found suitable for inclusion in breeding programmes due to their good performance. A better understanding of the genetic diversity present in the collection is necessary in order to optimize its use and maintenance. Previous studies on the genetic diversity present in different *B. rapa* collections have used molecular markers, proving that they are useful tools for the characterization of agricultural crop diversity (Persson *et al.* 2001; Zhao *et al.* 2005;

Ofori *et al.* 2008). The objectives of the present work were to assess the genetic diversity present in the *B. rapa* subsp. *rapa* collection maintained at Misión Biológica de Galicia, to establish genetic relationships among populations and to study the genetic structure of the collection using simple sequence repeats (SSRs).

MATERIALS AND METHODS

Plant material and DNA analysis

Eighty populations of *B. rapa* subsp. *rapa* collected in north-western Spain and the north of Portugal and kept at the Misión Biológica de Galicia (CSIC, Spain) Gene Bank were chosen for evaluation in the present study (Table 1). Populations from north-western Spain were collected throughout the region, in coastal and inland locations (Fig. 1). A previous study (Padilla *et al.* 2005) classified the 80 populations into different clusters, based on morphological and agronomical traits. Because populations are heterogeneous and highly variable, 20 individuals from each population were analysed. Plants were sown in seedbeds and, 40 days later, the fourth or fifth leaf of each individual were used for DNA extraction following the method of Liu & Whittier (1994) but without using microbeads. After an initial screening using 40 different SSRs, 18 SSRs that were polymorphic and

Fig. 1. Distribution of the populations analysed in north-western Spain.

independent were finally chosen to carry out the analysis (Table 2). Amplification of SSRs was performed using a PTC-100™ Thermal Cycler (MJ Research, Watertown, MA, USA). The amplification consisted of a denaturing step at 95 °C for 5 min followed by 35 denaturing cycles at 95 °C for 30 s, annealing at 56 °C for 30 s and elongation at 72 °C for 30 s. The programme ended with an extra elongation period of 10 min followed by a continuous cycle at 4 °C. Reactions were carried out with 50 ng of each primer, 0.6 unit of Taq polymerase (BioTaq, Kapa Biosystems), 200 µM each dNTP, 1× reaction buffer, 2.0 mM MgCl₂, 50 ng DNA template, in a final volume of 25 µl made up with distilled and autoclaved water. After amplification, SSR products were separated by electrophoresis on 0.6 g/ml non-denaturing acrylamide gels run in 1×TBE buffer. Gels were stained with Ethidium bromide, run for c. 2 h at 250 V and then visualized under UV light. Bands amplified by each SSR were sized by comparison with hyperladder IV (Bioline). Alleles were scored as present (1) or absent (0) for each SSR across populations. A band

was considered as present in a population if it was amplified in one individual of the 20 studied from the population.

Genetic diversity and genetic relationships

The number of alleles was computed for each SSR and each population. Average polymorphic information content was computed for each locus and expected heterozygosity average was computed for each population. A similarity matrix with presence-absence data was constructed using the NTSYS-PC version 2.1 (Rohlf 1998) based on the Dice coefficient, also known as the similarity coefficient of Nei & Li (1979). The Dice coefficient is computed as $2a/(2a+b+c)$, where a is the number of SSR bands shared by genotypes in each pairwise comparison and b and c are the number of SSR bands present in one genotype and not present in the other. Cluster analysis was performed using the unweighted pair group method with arithmetic averages (UPGMA) with the program NTSYS (Rohlf 1998). A cophenetic

Table 2. List of SSRs employed in this study, number of alleles and polymorphic information content

Primer pair*	Number of alleles	Polymorphic information content
BRMS-034	4	0.43
BRMS-043	3	0.56
BRMS-027	6	0.74
BRMS-007	9	0.78
BRMS-040	6	0.57
BRMS-051	3	0.31
BRMS-056	5	0.73
BRMS-006	8	0.80
BRMS-005	6	0.61
SSR Ra2-G09	5	0.68
SSR Ni2-F11	3	0.59
SSR Oi11-H06	3	0.42
FITO 008	4	0.65
FITO 035	3	0.50
FITO 036	3	0.33
FITO 043	4	0.52
FITO 057	4	0.28
FITO 063	3	0.57
Mean	4.5	0.56

* Primer sequences and annealing temperatures of primer pair starting with 'BRMS', 'SSR' and 'FITO' were obtained from Lowe *et al.* (2004), Suwabe *et al.* (2002) and Iniguez-Luy *et al.* (2008), respectively.

correlation was calculated to test for the goodness-of-fit between the similarity matrix obtained from the cluster and the original similarity matrix. Bootstrap values to ascertain the internal support of clusters were calculated with the software Win Boot (Yap & Nelson 1996).

Genetic structure

An analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) based on the dissimilarity matrix of pairwise individuals was performed using Arlequin version 3.2 (Excoffier *et al.* 2005) to assess the genetic structure of the *B. rapa* germplasm. This analysis partitioned the total SSR variation into within- and among-population variation components. The significance level for variance components was tested probabilistically by a permutation test. The overall *Fst* was computed with Arlequin version 3.2 and gene flow was estimated from *Fst* according to Nei (1973) as follows:

$$Nm = 0.25(1 - Fst)/Fst$$

Based on the genotype of the marker loci, individuals were assigned probabilistically into a given number of clusters inferred by the Bayesian approach of STRUCTURE (Pritchard *et al.* 2000). The number of clusters (*k*) chosen for modelling the data varied from 1

to 80. The likelihood of each *k* value *L(k)* was computed based on an initial burn-in period of 10000 interactions, followed by 10000 Monte Carlo interactions. The range of *k* values that showed the highest likelihoods (from *k* = 1 to *k* = 43) were run five different times. All runs used an admixture model with correlated allele frequencies and the parameter of individual admixture (alpha) was set to be the same for all the clusters. The accepted number of clusters (*k*) was identified using the procedure described by Evanno *et al.* (2005). Evanno *et al.* (2005), using simulated data, found that the distribution of *L(k)* did not show a clear mode for the true *k* value. They proposed an *ad hoc* quantity based on the second-order change of the likelihood with respect to *k* (Δk). The parameter $\Delta k = m(|L'|)/s[L(k)]$ is calculated as the mean of absolute values of the second-order change of the likelihood distribution divided by the standard deviation of likelihood. The modal value of this distribution is interpreted as the true number of clusters *k* (Evanno *et al.* 2005).

RESULTS

Genetic diversity and genetic relationships

The 18 SSRs detected a total of 83 different alleles across populations (Table 2). The number of alleles per locus ranged from 3 (several SSRs) to 9 (with BRMS 007), with a mean value of 4.5. The polymorphic information content ranged from 0.28 (using FITO 057) to 0.80 (using BRMS-006) (Table 2).

The genetic variation of each population was quantified by the number of alleles per locus and average heterozygosity (Table 1). The number of alleles per population ranged from 33 (MBG-BRS0123) to 57 (MBG-BRS0057), with a mean value of 48, and the expected heterozygosity ranged between 0.265 (MBG-BRS0123) and 0.536 (MBG-BRS0284) with a mean value of 0.455. The higher number of alleles and expected heterozygosity indicates the presence of a larger genetic variation; four populations: MBG-BRS0123, MBG-BRS0099, MBG-BRS0440 and MBG-BRS0461, showed the lowest level of genetic diversity and two populations, MBG-BRS0284 and MBG-BRS0139, showed the highest level of genetic diversity.

To clarify the relationships among populations a dendrogram based on the Dice coefficient, using the UPGMA method, was constructed (data not shown). Most of the populations were classified together for a distance of 0.82. Bootstrap analysis was used to evaluate the degree of support for clusters within the dendrogram (data not shown). Major clusters and subgroups within clusters are supported by low bootstrap values. The highest bootstrap values (>0.30) corresponded to clusters between pairs of populations; the remaining clusters showed support ≤ 0.30 . The

Table 3. AMOVA for 80 populations of *B. rapa*

Source of variation	D.F.	Sum of squares	Variance components	Proportion of variation	<i>P</i> value*
Among populations	79	1099	0.480	0.10	< 0.001
Within populations	1519	6560	4.318	0.90	
Total	1598	7659	4.798		

* Probability to obtain a random value greater than and equal to an observed value tested by 1023 permutations.

cophenetic correlation between the similarity matrix based on Dice coefficient and the similarity matrix obtained by the cluster was 0.80.

Genetic structure

Most of the variation was located at the within-population level (0.90). The source of variation among populations accounted for 0.10 of the variability (Table 3). The fixation index (*Fst*) for the populations analysed was 0.100 and the level of gene flow based on *Fst* was 2.25 migrants per generation.

To detect the population structure of the total sample, the Bayesian approach implemented in STRUCTURE (<http://pritch.bsd.uchicago.edu/structure.html>, verified 13 Jan 2010) was used. This program allows assignment of individuals to *k* clusters and determines the proportion of genome of each individual in each assigned cluster. The most probable *k* value, taking into account *L(k)* was 28 (Fig. 2). However, when Δk was computed, the most probable number of clusters supported by the 1600 individuals analysed was 1 (Fig. 2).

DISCUSSION

Genetic diversity and genetic relationships

The diversity found within *B. rapa* populations analysed in the present study was higher than in the work reported by Fu & Gugel (2009), who detected levels of heterozygosity from 0.120 to 0.197 in 10 cultivars of *B. rapa* subsp. *oleifera* analysed by SSR markers. The lower level of diversity found by Fu & Gugel (2009) can be explained because the populations analysed were related by origin. The heterozygosity found by Ofori *et al.* (2008) in three open-pollinated cultivars of *B. rapa* subsp. *oleifera* (ranging from 0.480 to 0.530) was similar to those found in some of the populations analysed in the present work.

There was a broad range of genetic diversity in the populations analysed. Populations also showed a high level of genetic diversity for morphological and agronomical traits in previous work (Padilla *et al.* 2005). Therefore, populations kept at the Gene Bank at Misión Biológica de Galicia offer good potential for further genetic improvement due to their high variability. Traditional varieties grown for years in

Fig. 2. (a) Distribution of *L(k)* for *k* = 1 to *k* = 43 based on an initial burn-in period of 10000 interactions, followed by 10000 Monte Carlo interactions and (b) distribution of Δk for *k* = 1 to *k* = 43 computed using the procedure described by Evanno *et al.* (2005).

north-western Spain have been selected both naturally and artificially for yield, resistance to pest and diseases and adaptation to the area where they are grown. They could be an important source of useful diversity for agronomic characteristics and resistance to diseases and pests for *Brassica* breeding programmes.

To study relationships among populations, a cluster analysis was carried out. Populations did not differ significantly since the majority of the populations were classified together. Clusters of populations were supported by low bootstrap values and the cophenetic correlation was moderate. An analysis of the genetic structure of the collection was carried out to study in detail the causes of this low level of differentiation.

Genetic structure

Most of the variability found in the populations analysed was due to the source of variation within populations. Previous studies on diversity within- and among-cultivar groups have been reported in *B. rapa*. Persson *et al.* (2001), using allozymes, reported 0.81 within-cultivar genetic diversity in turnip *B. rapa* from Northern Europe. High levels of within-population variation (0.90 and 0.83) were found in cultivars of *B. rapa* subsp. *oleifera* (Ofori *et al.* 2008; Fu & Gugel 2009). Zhao *et al.* (2005), applying AFLP markers, reported comparable genetic diversity among and within different cultivars belonging to different *B. rapa* crops, including leafy vegetables, oilseed, root vegetables and fodder collected world-wide.

The results presented here confirm the low differentiation among populations, which is not surprising for an open-pollinated species as *B. rapa*. The high genetic diversity within populations is required to avoid inbreeding and to enable a high yield since, as an obligate outcrossing crop, *B. rapa* shows a severe inbreeding depression. Examples of similar patterns can also be found in other open-pollinated crops such as maize (Reif *et al.* 2003) and sugar beet (Novakova *et al.* 2008).

Fst is a measure of the extent of genetic differentiation among subpopulations and can range from 0 (no differentiation) to 1 (complete differentiation). As expected for an outcrossing crop, a low overall differentiation among populations was observed. Gene flow is the movement of genes within and between populations. Generally, a single migrant per generation among idealized populations would be sufficient to prevent complete differentiation (Wright 1969). In the present study, the level of gene flow based on *Fst* was 2.25 migrants per generation, which confirms the supposition of little genetic differentiation among populations. Gene flow in the collection of *B. rapa* subsp. *rapa* would occur naturally by means of migration (movement of pollen or seed carriers) or artificially by means of interchange or mixture of seed by growers.

The most probable *k* value computed with the method proposed by Evanno *et al.* (2005) does not agree with the one obtained by taking into account the distribution of *L(k)*. Evanno *et al.* (2005) have

demonstrated that *L(k)* does not show a clear mode for the true *k* value; however, the parameter Δk , taking into account changes of the likelihood distribution and the standard deviation of likelihood, is more reliable. In this case, although *L(k)* of *k* = 1 was low, its standard error was very small, then its Δk was by far the highest value of the distribution, and therefore it can be concluded that all populations belong to the same cluster.

Using these data on SSRs, the 80 populations were clustered together independently of their geographical origin or morphological resemblance. This last result contrasts with Padilla *et al.* (2005), who classified all the accessions of the same *B. rapa* subsp. *rapa* collection into five clusters, based on morphological and agronomical traits, related to the potential use of the crop: vigorous plants for turnip leaf production, plants with a high number of secondary stems for turnip inflorescences and plants with the root thickened for turnip production. The lack of agreement between the classifications could be partly due to the fact that differences in the use of the plant are determined by few genes and that the number of SSR markers used in the present work is not enough to detect their effect.

After studying the *B. rapa* subsp. *rapa* kept at Misión Biológica de Galicia collection it can be concluded that it has a broad range of genetic diversity. Most of the variability was found within populations, due to: high rates of allogamy in the species, migration, interchange of seed or mixing of seed by growers. This means that populations are not differentiated, and therefore they can be considered as samples from a highly variable metapopulation that can be used for *B. rapa* breeding programmes.

As a result of the present work, the new knowledge on the genetic diversity and genetic structure of this collection will be useful for its utilization and management.

The authors thank the invaluable work carried out by Rosaura Abilleira, Eva Fernández, Susana Calvo and Elena Zubiaurre. Marta Francisco and Margarita Lema acknowledge their grants from the I3P program (CSIC, Spain). This research was supported by project RF-2006-00028-00-00 and the Excma. Diputación Provincial de Pontevedra, Spain.

REFERENCES

- EVANNO, G., REGNAUT, S. & GOUDET, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**, 2611–2620.
- EXCOFFIER, L., SMOUSE, P.E. & QUATTRO, J.M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479–491.
- EXCOFFIER, L., LAVAL, G. & SCHNEIDER, S. (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1**, 47–50.
- FU, Y.-B. & GUGEL, R.K. (2009). Genetic variability of Canadian elite cultivars of summer turnip rape (*Brassica rapa* L.) revealed by simple sequence repeat markers. *Canadian Journal of Plant Science* **89**, 865–874.

- GÓMEZ-CAMPO, C. & PRAKASH, S. (1999). Origin and domestication. In *Biology of Brassica Coenospecies* (Ed. C. Gómez-Campo), pp. 33–58. Amsterdam, The Netherlands: Elsevier.
- INIGUEZ-LUY, F. L., VOORT, A. V. & OSBORN, T. C. (2008). Development of a set of public SSR markers derived from genomic sequence of a rapid cycling *Brassica oleracea* L. genotype. *Theoretical and Applied Genetics* **117**, 977–985.
- LIU, Y. G. & WHITTIER, R. F. (1994). Rapid preparation of megabase plant DNA from nuclei in agarose plugs and microbeads. *Nucleic Acids Research* **22**, 2168–2169.
- LOWE, A. J., MOULE, C., TRICK, M. & EDWARDS, K. J. (2004). Efficient large-scale development of microsatellites for marker and mapping applications in *Brassica* crop species. *Theoretical and Applied Genetics* **108**, 1103–1112.
- NEI, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Science of the United States of America* **70**, 3321–3323.
- NEI, M. & LI, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Science of the United States of America* **76**, 5269–5273.
- NOVAKOVA, K., HAMOUZ, P., HOLEC, J. & SOUKUP, J. (2008). Genetic diversity of Beta-complex assessed by RAPD markers. *Journal of Plant Diseases and Protection* **21** (Special Issue), 437–440.
- OFORI, A., BECKER, H. C. & KOPISCH-OBUCH, F. J. (2008). Effect of crop improvement on genetic diversity in oilseed *Brassica rapa* (turnip-rape) cultivars, detected by SSR markers. *Journal of Applied Genetics* **49**, 207–212.
- PADILLA, G., CARTEA, M. E., RODRÍQUEZ, V. M. & ORDÁS, A. (2005). Genetic diversity in a germplasm collection of *Brassica rapa* subsp. *rapa* L. from northwestern Spain. *Euphytica* **145**, 171–180.
- PERSSON, K., FÄLT, A.-S. & VON BOTHMER, R. (2001). Genetic diversity of allozymes in turnip (*Brassica rapa* L. var *rapa*) from the Nordic area. *Hereditas* **134**, 43–52.
- PRITCHARD, J. K., STEPHENS, M. & DONNELLY, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.
- REIF, J. C., MELCHINGER, A. E., XIA, X. C., WARBURTON, M. L., HOISINGTON, D. A., VASAL, S. K., SRINIVASAN, G., BOHN, M. & FRISCH, M. (2003). Genetic distance based on simple sequence repeats and heterosis in tropical maize populations. *Crop Science* **43**, 1275–1282.
- ROHLF, F. J. (1998). *NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System. Version 2.1*. New York: EXETER software.
- SUWABE, K., IKETANI, H., NUNOME, T., KAGE, T. & HIRAI, M. (2002). Isolation and characterization of microsatellites in *Brassica rapa* L. *Theoretical and Applied Genetics* **104**, 1092–1098.
- WRIGHT, S. (1969). *Evolution and the Genetics of Populations, 2: The Theory of Gene Frequencies*. Chicago, IL: University of Chicago Press.
- YAP, I. V. & NELSON, R. J. (1996). *Winboot: a Program for Performing Bootstrap Analysis of Binary Data to Determine the Confidence Limits of UPGMA-based Dendograms*. Discussion Paper Series No. 14. Manila, The Philippines: IRRI.
- ZHAO, J., WANG, X., DENG, B., LOU, P., WU, J., SUN, R., XU, Z., VROMANS, J., KOORNNEEF, M. & BONNEMA, G. (2005). Genetic relationships within *Brassica rapa* as inferred from AFLP fingerprints. *Theoretical and Applied Genetics* **110**, 1301–1314.