Heredity **78** (1997) 403–409 *Received 4 April 1996*

Assessment of genetic relationships among sexual and asexual forms of *Allium cepa* using morphological traits and RAPD markers

MAUD LE THIERRY D'ENNEQUIN*, OLIVIER PANAUD, THIERRY ROBERT AND AGNÈS RICROCH

Laboratoire Evolution et Systématique, bâtiment 362, Université Paris-Sud. URA CNRS 2154, 91405 Orsay cedex, France

The species *Allium cepa* includes two major crops on the basis of morphological traits and typical reproduction mode: sexually reproduced biennial onions and vegetatively propagated perennial shallots which rarely flower. In addition, the seed-propagated shallot, a recently released variety with intermediate phenotype for life history, has been described and used by breeders. A joint analysis using molecular markers (random amplified polymorphic DNA, RAPD) and morphological characters was undertaken for the assessment of genetic diversity and crop classification among *Allium cepa* genotypes. A morphological study describing growth and development was carried out on a nested sampling (34 accessions) from two geographical origins (European or tropical) and of different plant status (improved varieties or local ecotypes). Four primers generated 24 reproducibly scorable DNA bands which gave individual fingerprinting for representative accessions of both onions and shallots. Our results indicate that the seed-propagated shallot is more closely related to onions than to vegetatively propagated shallots and, moreover, reveal a geographical structure of genetic diversity. The evolutionary significance of these data is discussed.

Keywords: *Allium cepa*, diversity, onion, RAPD, reproductive strategy, shallot.

Introduction

The domestication process of *Allium cepa* is very ancient, as shown by the presence of onions in Egypt 5000 years ago. According to Zeven & Zhukovsky (1975), there is either a single centre of origin for both onions and shallots located in central Asia or two different centres located in the same area. Nowadays selection and isolated cultivation have produced a large spectrum of locally adapted varieties with respect to daylength response for bulbing. Local ecotypes display a high genetic variability for traits such as photoperiod, precocity of bulbing and bulb dormancy (Rouamba *et al.*, 1993). However, the influence of geographical distribution in respect of daylength response on the structure of genetic diversity is insufficiently studied.

The species *A. cepa* displays different reproductive modes: sexual reproductive onions (allogamous and protandrous) and vegetatively propagated shallots. Recent classifications of *A. cepa* suggest a partition of the species into three botanical groups (Hanelt *et al.*, 1992): the Common Onion group, constituted by sexual reproductive onions, the Aggregatum group, formed with vegetatively propagated shallots, and the Proliferum group, which presents vegetatively propagated onions producing bulblets (topset).

Asexual shallots have been placed in the species *A. ascalonicum*; however, its interfertility with *A. cepa* (Atkin, 1953) was the reason for considering both as the same species (*A. cepa*). The existence of those two interfertile forms suggests the presence of an evolutionary continuum between a sexual form and a vegetatively reproduced form selected against flowering during the domestication process. Onions and asexual shallots can be differentiated by morphological characters (single, large bulb for onions vs. bulblets for shallots), by their reproductive mode (sexual reproduction vs. asexual reproduction) and by their life cycle (biennial for onions vs. perennial for shallots).

Taxonomy and genetic diversity within the genus *Allium* have already been studied by isozymes, rDNA, restriction fragment length polymorphism

*Correspondence. E-mail: lethier@psisun.u-psud.fr

(RFLP) and RAPD markers. Studies using isozymes have measured genetic variability of between- and within-population variation (Peffley & Orozco-Castillo, 1987; Rouamba *et al.*, 1993). Taxonomic studies carried out with RFLP markers revealed high differentiation between species, especially for species belonging to the section *Cepa* (Bradeen & Havey, 1995). RFLP markers have been used at an intraspecific level in order to analyse genetic similarities between onions with different kinds of daylength response (Bark & Havey, 1995). Genetic relationships between *A. cepa* and *A. fistulosum* were reported by Ricroch *et al.* (1992) using *in situ* hybridization of rDNA sequences.

RAPD markers are useful for determining the distribution of genetic variability within and among taxonomic units and have been applied for this purpose in different species, especially on clones (Castiglione *et al.*, 1993). They have been used in *A. sativum* (Maaß & Klaas, 1995) and in *A. cepa* (Wilkie *et al.*, 1993; Bohanec *et al.*, 1995).

In this study, we assessed the efficiency of using chosen morphological characters and RAPD markers to examine the distribution of genetic diversity among several accessions of sexual and asexual forms of *A. cepa* representing a wide geographical range. Moreover, we addressed the question of whether the seed-propagated shallot belongs to the onion or shallot groups.

Materials and methods

Growth conditions

Seeds of 19 accessions of onions and bulblets of 21 shallot accessions were planted between 2 and 10 February and cultivated under standard conditions in greenhouses at Paris-Sud University (France) (Table 1). During the period of growth and development, the mean minimum and maximum temperature ranged from 15 to 32°C. For each form, ecotypes (coming from germplasm collections) and improved varieties (currently under selection or commercial varieties) from tropical and European origins were chosen. The seed-propagated shallot was the French commercial variety 'Cuisse de Poulet', cultivated in the western region of France. Both seeds and bulblets were cultivated.

Morphological analysis

Plant material The number of analysed individuals per accession was chosen according to reproductive mode. For sexually reproduced onions 10 individuals per accession were analysed. For vegetatively propagated shallots three individuals per clone were studied. Among 21 shallot accessions, 14 accessions were used in the morphological study. No morphological data were recorded on seven accessions that flowered. For the seed-propagated shallot, 10 individuals (both from seeds and bulblets) were analysed.

Characteristics describing growth and development listed in Table 2 were chosen because they have been shown to be valuable descriptors of onion and shallot life history (Messiaen *et al.*, 1993). Every week, bulb and neck diameters of onions were measured during the development cycle in order to determine a bulb ratio. When maximal bulb diameter is twice minimal neck diameter (R2), it indicates the onset of bulbing (Brewster, 1977). The number of days from sowing to R2 was calculated. Leaf number of onions and shallots was scored every week until harvesting. The other characteristics were determined at maturity. A plant was determined as mature when 50 per cent of leaves wilted and were recumbent on the ground. At harvest onion bulbs and shallot bulblets were dried at 80°C for 48 h and the dry matter content was measured.

Data analysis The data recorded from the 33 accessions of onions and vegetatively propagated shallots were submitted to multivariate statistical analysis. The combined procedure described by Sarr & Pernès (1988) was used. This procedure involves a principal component analysis, a clustering algorithm using the Euclidian distance calculated from the data matrix, and a discriminant analysis. It allows an accurate description of phenotypic variation and its organization within the gene pool studied.

Molecular study

Plant material Eight European and tropical onion accessions were chosen. They represented the daylength response spectrum of local ecotypes and improved varieties. Per accession, 1–10 individuals were scored for RAPD analysis. This sampling scheme ensured representation of a large geographical and ecotypic range. For shallots, the 14 accessions representative of ecotypes and commercial clones were chosen. Because the shallot sample contained only 14 accessions (17 genetic individuals), the sample size was too small to assess the geographical distribution of the genetic variability. For the seed-propagated shallot, eight of the 10 individuals tested in the morphological analysis were examined.

Table 1 Plant status and geographical origin of *Allium cepa* accessions studied in a morphological analysis (M) and in a molecular study (RAPD)

Onions			
Ecotypes			
European origin			
Rosé de Roscoff 33A (France)[a], OP*	**O-01**	M	RAPD
Rosé de Roscoff 33B (France)[a], OP	**O-02**	M	
Raïolette des Cévennes (France)[a], OP	**O-03**	M	
Museau de lièvre 47A (France)[a], OP	**O-04**	M	RAPD
Tropical origin			
Tintilou (Burkina-Faso)[b], OP	**O-05**	M	RAPD
4Ni (Niger)[b], OP	**O-06**	M	
Improved varieties			
European origin			
Jaune hâtif de Valence (Spain)[c], OP	**O-07**	M	
Jaune espagnol (Spain)[c], OP	**O-08**	M	RAPD
Rouge espagnol (Spain)[d], OP	**O-09**	M	
Blanc de Paris (France)[c], OP	**O-10**	M	
Auxone (France)[e], OP	**O-11**	M	RAPD
Tropical origin			
Short day hybrid (USA)[f], F_1**	**O-12**	M	
Violet de Galmi Ma027 (Mauritania)[g], OP	**O-13**	M	RAPD
Violet de Galmi 1634 (Ivory Coast)[g], OP	**O-14**	M	RAPD
Violet de Galmi No5 (Senegal)[g], OP	**O-15**	M	
Short day white (USA)[f], OP	**O-16**	M	
Rio Solo (USA)[h], F_1	**O-17**	M	RAPD
Rio Enrique (USA)[h], F_1	**O-18**	M	
Rio Radji red (USA)[h], F_1	**O-19**	M	
Vegetatively propagated shallots			
Ecotypes			
European origin			
E2 (France)[i]	**S-01**	M	RAPD
E9 globe (France)[i]	**S-02**	M	
E14 (France)[i]	**S-03**	M	
E15 long (France)[i]	**S-04**	M	RAPD
E18 globe (France)[i]	**S-05**	M	
Tropical origin			
Guinée (Guinea)[j]	**S-06**	M	
Abidjan (Ivory Coast)[j]	**S-07**	M	
Brazzaville (Congo)[j]	**S-08**	M	RAPD
OAEG (Guinea)[j]	**S-09**	M	
Improved varieties			
European origin			
E1 (France)[i]	**S-10**		RAPD
E3 (France)[i]	**S-11**	M	RAPD
E4 (France)[i]	**S-12**		RAPD
E7 (France)[i]	**S-13**		RAPD
E10 globe (France)[i]	**S-14**		RAPD
E11 globe (France)[i]	**S-15**	M	RAPD
E13 long and 'grise'(France)[i]	**S-16**	M	RAPD
E16 experimental, globe (France)[i]	**S-17**		RAPD
E17 globe (France)[i]	**S-18**		RAPD
E19 experimental, globe (France)[i]	**S-19**		RAPD
Echalote grise (France)[k]	**S-20**	M	RAPD
Tropical origin			
Oignon blanc (Ivory Coast)[b]	**S-21**	M	
Seed-propagated shallots			
European origin			
Cuisse de poulet (France)[l], OP	**SP-1**	M	RAPD

The origins of accessions are indicated in brackets.
[a]Ecole National Supérieure d'Horticulture de Versailles (France); [b]Institut National d'Etudes en Recherche Agronomique (Burkina-faso); [c]Clause (France); [d]Vilmorin (France); [e]Coopd'or (France); [f]d palmers seed (USA); [g]Tropicasem (Senegal); [h]Rio Colorado (USA); [i]Institut National de Recherche Agronomique de Ploudaniel (France); [j]Centre de coopération Internationale en Recherche Agronomique pour le Développement de la Réunion (France); [k]Rungis market (France); [l]Les jardiniers d'Anjou et du Poitou à Allonnes (France).
*Open pollinated; **F_1 hybrid.

Table 2 List of traits measured on onions and vegetatively propagated shallots

Onions	Shallots
Leaf number (LN)	Leaf number (per cluster of bulblets) (LN)
Leaf number at R2 (LNR2)	Bulblet diameter (BD)
Bulb diameter (BD)	Bulblet height (BH)
Bulb height (BH)	Bulblet fresh matter content (BFMC)
Fresh matter content (FMC)	Bulblet dry matter content (BDMC)
Dry matter content (DMC)	Bulblet plate length (BPL)
Plate length (PL)	Bulblet number (BN)
Axillary buds number (ABN)	
Days number from sowing to R2 (DNR2)	

RAPD amplification

DNA was isolated from fresh leaf material (0.7 g) by a CTAB method based on that of Saghai-Maroof *et al.* (1984). One of four 10-mer oligonucleotides: OPA-04 (5′-AATCGGGCTG-3′), OPA-19 (5′-CAAACGTCGG-3′), OPA-20 (5′-GTTGCGATCC-3′) and OPB-11 (5′-GTAGACCCGT-3′) (Operon technologies Inc., Alameda, California, USA) were used for each amplification. Conditions reported by Bohanec *et al.* (1995) were improved. Each reaction (25 μL) consisted of 1 mM of $MgCl_2$, 4 mM each of dATP, dCTP, dGTP, dTTP (Boehringer-Manheim, Germany), 400 nM primer, 0.5 U of *Taq* DNA polymerase (Appligene-Oncor, France), 1 × reaction buffer (1 mM $MgCl_2$, 20 mM Tris HCl pH 8.0, ethylenediaminetetraacetic acid (EDTA) 1 mM, dithiothreitol 1 mM, glycerol 50 per cent). The reaction mix was overlaid with a drop of mineral oil and incubated in a thermal cycler (thermal cycler 480, Perkin Elmer-Cetus, USA) programmed as follows: 48 cycles of 1 min denaturation at 94°C, 1 min annealing at 37°C and 1.5 min extension at 72°C, followed by final extension at 72°C followed by a cooling at 4°C. Tubes containing all reaction products except template DNA were used as control. Ten millilitres of amplification products were electrophoresed in 3 per cent agarose gels in 0.5 × TBE. We used ϕX DNA digested with *Hae*III as a molecular weight marker.

Data analysis

Each amplification was repeated three times. The visible and reproducible bands were scored as present (1) or absent (0). Similarity indices (F) from Jaccard (1908) and Nei & Li (1979) were calculated. Genetic distances (1-F) were used to build a dendrogram according to the UPGMA clustering procedure. Because results using the two indices were similar, only the dendrogram based on the Nei and Li distance is shown. The Boolean matrix was also submitted to factorial correspondence analysis (Morrison, 1990). This multivariate procedure is similar to a principal component analysis except for computation of coordinates along axes which is based on a χ^2 distance.

Results

Morphology and life history

The life history of the seed-propagated shallot was observed from sowing seeds or from planting bulbs. From seed, one large bulb was obtained which rapidly divided to produce a cluster of small bulbs. When one bulb was planted it further divided to form a cluster of small bulbs, followed by flowering in the second year.

The principal component analysis based on morphological characters (data not shown) suggested that phenotypic diversity was organized in two and three major clusters for onions and vegetatively propagated shallots, respectively. This structure was confirmed by a clustering algorithm followed by a discriminant analysis which allowed a final classification (Table 3).

In onions, the most discriminant variables were bulb fresh matter content (FMC) and bulb height (BH), both descriptors of bulb yield. Moreover, BH is a morphological character reflecting selection for bulb shape. Geographical distinction was less obvious for vegetatively propagated shallots than for onions. Three clusters were discriminated by three major variables: bulblet plate length (BPL), leaf number (LN) and bulblet fresh matter content (BFMC). Clusters 1 and 2 included all the European accessions (both improved varieties or local ecotypes); the first one was composed of accessions that presented a very good yield, and the second one

was formed by low yield accessions which have high values for variables depicting life history (LN, BPL). Cluster three contained all the tropical accessions. They were characterized by low yield values similar to the values of cluster 2. Variables (LN and BFMC) involved in the discriminant analysis of vegetatively propagated shallots describe carbohydrate allocation from leaves to storage organs. Concerning the seed-propagated shallot, we noticed the emergence of both seeds and bulblets (topsets) within umbels.

Differentiation of sexual and asexual forms based on RAPD markers

The choice of the four primers in this present study was based on the polymorphism revealed in previous studies on *Allium* species (Wilkie *et al.*, 1993; Bohanec *et al.*, 1995). These four primers generated a total of 24 reproducible bands scored for statistical analysis. An example of patterns using primer OPA 19 for different accessions of onion shows polymorphism within and between accessions (Fig. 1). RAPD analysis did not identify any band specific to one of the three forms (onions, vegetatively propagated shallots or the seed-propagated shallot). However, some bands were more frequent in one form than in another.

The dendrogram (Fig. 2) and the two factorial component analyses (data not shown) from analysis of RAPD bands showed a differentiation between the 'echalote grise' (S-16 and S-20), the vegetatively propagated shallots and the onions. The seed-propagated shallot is closer to onions than to vegetatively propagated shallots. The differentiation is mainly in

Table 3 Results of discriminant analysis for onions and shallots based on morphology and life cycle traits

	Onions	Shallots
Cluster number	2	3
% of well-classified individuals	100	100
Discriminant power of the canonical variables	0.93	0.95; 0.73
The more discriminant variables	FMC, BH*	BPL, LN, BFMC*
Cluster 1	O-01, O-02, O-03, O-04, O-09, O-10, O-11	S-01, S-02, S-03, S-15, S-16
Cluster 2	O-05, O-06, O-07, O-08, O-12, O-13, O-14, O-15, O-16, O-17, O-18, O-19	S-04, S-11, S-05, S-20
Cluster 3		S-06, S-07, S-08, S-09, S-21

*See Table 2 for abbreviations.

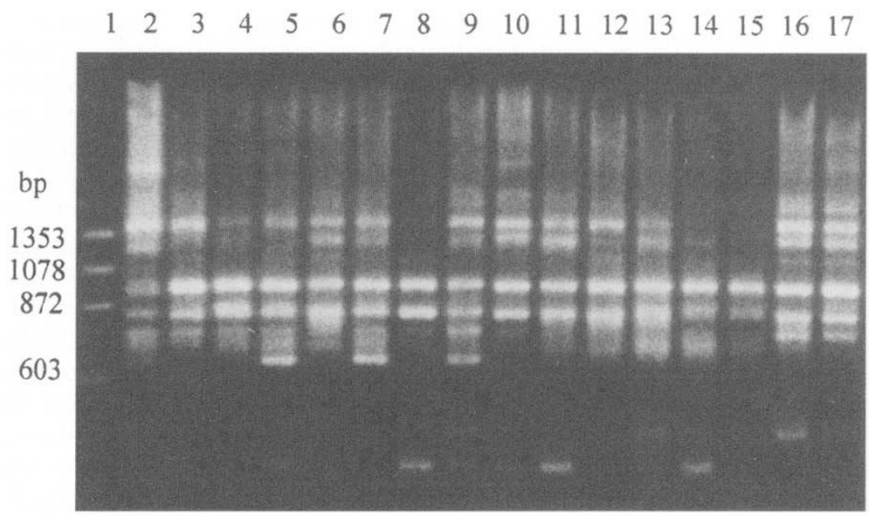

Fig. 1 Patterns obtained with one primer (OPA 19) for individuals of three accessions of onions. Lane 1: ladder; lanes 2–6: DNA from five individuals of accession 'Jaune espagnol'; lanes 7–11: DNA from five individuals of accession 'Violet de Galmi 1634'; lanes 12–17 from six individuals of accession 'Violet de Galmi Ma027'.

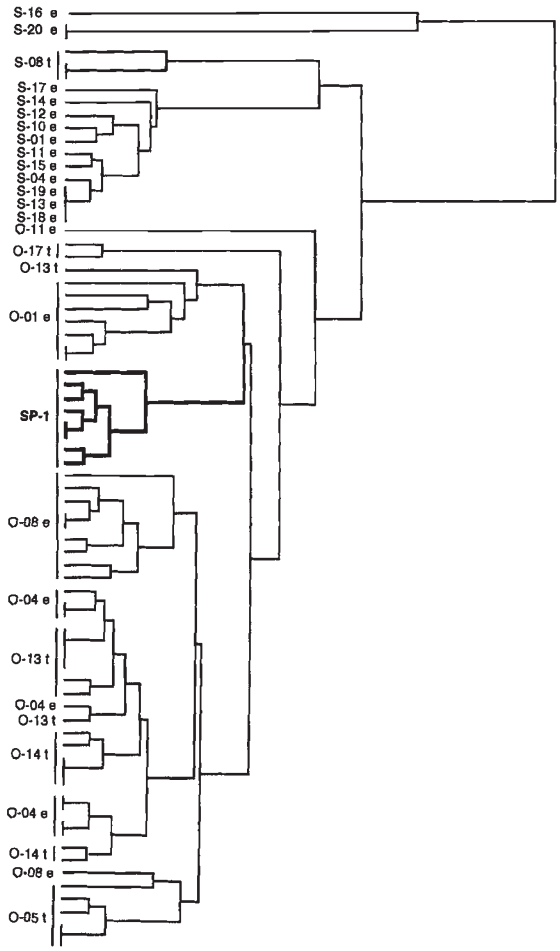

Fig. 2 Dendrogram showing the differentiation of onions, seed- and vegetatively propagated shallots (see Table 1 for accession details) built based on the Nei and Li distance (e, european origin; t, tropical origin).

relation to the reproductive mode; but within each form differentiation by geographical origin (tropical or European) was observed.

Discussion

The morphological study showed that the seed-propagated shallot constitutes an intermediate form with life history of both sexual and asexual forms. The life cycle of the seed-propagated shallot is biennial and characterized by bulblet (topsets) production (similar to vegetative growth of vegetatively propagated shallots) followed by seed production (similar to onions). The presence of a transient stage in its life history characterized by the formation of a single large bulb is not comparable to the onset of bulbing of onions. Consequently, the onset of bulbing determined by the bulb ratio (R2) could not be scored. The sexual characteristic of its life history prevented any comparison with the vegetative reproduction of the asexual form. However, this first part of the study confirmed geographical differentiation both for onions and vegetatively propagated shallots. Within onions the two clusters reflected European and tropical origins, with the exception of two European varieties ('Jaune hâtif de Valence' and 'Jaune espagnol') within the tropical cluster. The latter are short-day varieties with a low daylength requirement for bulbing that can be cultivated in tropical areas and in southern Europe. Differences between clusters probably reflect variation in selection for yield characteristics such as bulb shape. A similar phenomenon was observed within the asexual form, which may have either a large number of small bulblets or a small number of large bulblets. Concerning the seed-propagated shallot, the presence of bulblets (topsets) was probably caused by stressed growth conditions in greenhouses. This reproductive strategy is a trait observed in many wild taxa such as *A. vineale*, rarely in the Common Onion group but still frequently in the Proliferum group.

The use of neutral markers allowed the seed-propagated shallot to be compared with onions and vegetatively propagated shallots. Not surprisingly, the dendrogram (Fig. 2) showed that differentiation in relation to reproductive mode was more ancient than the geographical differentiation. The 'echalote grise' (S-16 and S-20) never flowers in natural conditions, although flowering can be induced by severe stress. Its divergence from the other vegetatively propagated shallots has already been suggested from morphological characteristics such as bulb skin, umbel morphology and root type (Messiaen *et al.*, 1993). Our results are in agreement with this observation. The molecular study suggests that the seed-propagated shallot should be classified in the onion cluster. Furthermore the dendrogram revealed that this sexual form was closer to European onions than to tropical ones.

The geographical differentiation found in the morphological study was confirmed by RAPD polymorphism. Rouamba (1992) has proposed that from the primary centre of diversity located in central Asia two independent pathways of migration to Europe and to Africa led to adaptive radiation of onions. If this hypothesis is valid, our results show that gene flow has probably occurred between European and tropical gene pools after their initial differentiation.

Further studies on the diversity found in wild species (such as *A. pskemense* or *A. vavilovii)* will

help to determine whether the domestication process of *A. cepa* has led to a decrease in genetic diversity through bottleneck events.

Acknowledgements

We thank Professor A. Sarr, Professor M. Klaas, Dr H.I. Maaß and Dr J. Shykoff for valuable comments on the manuscript. We gratefully acknowledge Dr R. Kahane, Dr F. Cohat, Dr A. Rouamba, Mr E. Gorin, Mr O. David and Mrs C. Desfeux for kindly providing plant material.

References

ATKIN, J. D. 1953. *Genetic and Cytological Studies of the Hybrid* Allium cepa × Allium ascalonicum. Ph. D. thesis, University of California (Davis), CA.

BARK, O. H. AND HAVEY, M. J. 1995. Similarities and relationships among populations of the bulb onion as estimated by nuclear RFLPs. *Theor. Appl. Genet.*, **90**, 407–414.

BOHANEC, B., JAKSE, M. AND JAVORNIK, B. 1995. Studies of gynogenesis in onion (*Allium cepa* L.): induction procedures and genetic analysis of regenerants. *Plant Sci.*, **104**, 215–224.

BRADEEN, J. M. AND HAVEY, M. J. 1995. Restriction fragment length polymorphisms reveal considerable nuclear divergence within a well-supported maternal clade in *Allium* section *Cepa* (Alliaceae). *Am. J. Bot.*, **82**, 1455–1462.

BREWSTER, J. L. 1977. The physiology of onion. *Hort. Abstr.*, **47**, 17–23.

CASTIGLIONE, S., WANG, G., DAMIANI, G., BANDI, C., BISOFFI, S. AND SALA, F. 1993. RAPD fingerprints for identification and for taxonomic studies of elite poplar (*Populus* spp.) clones. *Theor. Appl. Genet.*, **87**, 54–59.

HANELT, P., SCHULTZE-MOTEL, J., FRITSCH, R., KRUSE, J., MAß, H. I., OHLE, H. AND PISTRICK, K. 1992. Infrageneric grouping of *Allium* – the Gatersleben approach. In: Hanelt, P., Hammer, K. and Knüpffer, H. (eds) *The Genus Allium–Taxonomic Problems and Genetic Resources, Proceedings of an International Symposium*, pp. 107–123. Gatersleben.

JACCARD, P. 1908. Nouvelles recherches sur la distribution florale. *Bull. Soc. Vaud. Sci. Nat.*, **44**, 223–270.

MAAß, H. I. AND KLAAS, M. 1995. Infraspecific differentiation of garlic (*Allium sativum* L.) by isozymes and RAPD markers. *Theor. Appl. Genet.*, **91**, 89–97.

MESSIAEN, C. M., COHAT, J., LEROUX, J. P., PICHON, M. AND BEYRIES, A. 1993. In: INRA (ed.) *Les Allium Alimentaires Reproduits par Voie Végétative*, pp. 195–198. Paris.

MORRISON, D. F. 1990. *Multivariate Statistical Methods*, 3rd edn. McGraw-Hill Publishing Company, New York.

NEI, M. AND LI, W. H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 5267–5273.

PEFFLEY, E. B. AND OROZCO-CASTILLO, C. 1987. Polymorphism of isozymes within plant introductions of *Allium cepa* L. and *Allium fistulosum* L. *HortSci.*, **22**, 956–957.

RICROCH, A., PEFFLEY, E. B. AND BAKER, R. J. 1992. Chromosomal location of rDNA in *Allium*: *in situ* hybridization using biotin- and fluorescein-labelled probe. *Theor. Appl. Genet.*, **83**, 413–418.

ROUAMBA, A. 1992. *Analyse conjointe par les marqueurs agro-morphologiques et les allozymes de la diversité génétique de populations d'oignon* (Allium cepa *L.) d'Afrique de l'Ouest.* M. Sc. thesis, University of Paris VI.

ROUAMBA, A., RICROCH, A. AND SARR, A. 1993. Collecting onions germplasm in west Africa. *FAO/IBPRG Plant Genetic Resources Newsl.*, **94/95**, 15–17.

SAGHAI-MAROOF, M. A., SOLIMAN, K. M., JORGENSEN, R. A. AND ALLARD, R. W. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 8014–8018.

SARR, A. AND PERNÈS, J. 1988. Analyses multivariées de descendances de rétrocroisements de distorsions de ségrégation de caractères quantitatifs chez le mil (*Pennisetum typhoides* (Burm.)Stapf et Hubb.). *Genome*, **30**, 411–422.

WILKIE, S. E., ISAAC, P. G. AND SLATER, R. J. 1993. Random amplified DNA (RAPD) markers for genetic analysis in *Allium*. *Theor. Appl. Genet.*, **86**, 497–504.

ZEVEN, A. C. AND ZHUKOVSKY, P. M. 1975. *Dictionary of Cultivated Plants and their Centres of Diversity*, pp. 219–244. Centre for Agricultural Publishing and Documentation, Wageningen.