Theor Appl Genet (2000) 101:153–164 © Springer-Verlag 2000

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Onion microsatellites for germplasm analysis and their use in assessing intra- and interspecific relatedness within the subgenus *Rhizirideum*

Received: 8 October 1999 / Accepted: 3 November 1999

Abstract We have identified a set of informative STMS markers in onion (*Allium cepa* L.) and report on their application for genotyping and for determining genetic relationships. The markers have been developed from a genomic library enriched for microsatellites. Integrity of the microsatellite polymorphism was confirmed by amplicon sequencing. The microsatellite genotypes of 83 onion accessions and landraces from living onion collections were compared. As few as four primer pairs were sufficient to assign unique microsatellite patterns to the 83 accessions. Some of the microsatellite markers can be used for interspecific taxonomic analyses among close relatives of *Allium cepa*. Generally, our data support and extend results obtained from recently performed analyses using ITS, RAPD and morphology.

Key words *Allium* · Onion · Genotyping · Germplasm analysis · Microsatellite · Simple sequence repeat

Introduction

In the last decade, microsatellites have been widely used as genetic markers in plants, man, animals and fungi. The increasing interest in this marker for a broad range of applications is based on a high degree of intraspecific polymorphism, codominant genetics and its greater reliability and reproducibility compared to other molecular DNA markers (Jones *et al.* 1997; Powell *et al*. 1996). Marker-assisted breeding, resistance gene tagging and linkage mapping (Maughan *et al.* 1995; Taramino and Tingey 1996; Weising *et al.* 1998), diversity studies and genotyping in plants and plant pathogenic fungi (Neu *et al.* 1999; Geistlinger *et al*.1997; Kijas *et al.* 1995; Röder *et al.* 1998; Weising et *et al.* 1996; Smulders *et al*. 1997) and diagnostic procedures such as the detection of pathogen resistance genes (Blair and McCouch 1997; Fahima *et al*. 1998; Korzun *et al*.1998; Mudge *et al.* 1997) and agronomic traits (Xiao *et al*. 1996) represent important microsatellite applications in crop plants.

Some major crops within the genus *Allium* L. are bulb onion, shallot and chives. Several landraces and accessions have been selected and bred from wild species within the subgenus *Rhizirideum* of the genus, and these are presently grown in temperate to tropical climates on all continents. The genus has been extensively surveyed by means of morphological data (Hanelt et al. 1992), and various molecular studies have been undertaken (for an extensive review, see Klaas 1998). Most of these have focused on interspecific analyses, and only a few using random amplified polymorphic DNA (RAPD) (Wilkie *et al*. 1993; Bradeen and Havey 1995) or nuclear restriction fragment length polymorphism (RFLP) markers (Bark and Havey 1995) dealt with genetic analyses within the crop species *Allium cepa* L.

Intraspecific relationships in onion are still insufficiently resolved. The onion genome is large compared to those of most angiosperms (17.9 pg of DNA per 1C nucleus, Bennet and Smith 1991) and has a very high proportion of repetitive DNA: one telomeric satellite covers 4% of the genome(Barnes *et al*. 1988), and reassociation kinetic experiments have revealed 41% of the onion genome to have a mean repetition frequency of 21,600, another 36% with a mean repetition frequency of 225 and only 6% are single-copy domains. The remainder was not detectable using this method (Stack and Comings 1979). Both genome size and the high content of repetitive sequences cause difficulties and inconsistent results during marker development and application in *Allium*. This was reflected in the weak and ambiguous signals obtained from onion samples when DNA from various crops was probed with several microsatellite oligonucleotides (Sharon *et al*. 1995). Still there is a demand for

Communicated by R. Hagemann

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onion microsatellite markers, since redundancy and low reproducibility hampers sound statistical analyses of multilocus and dominant marker data. In onion this has only partially been overcome by using single copy probes for nuclear RFLP analysis.

Materials and methods

Marker development

The procedure used to develop sequence-tagged microsatellite (STMS) markers has been comprehensively described elsewhere (Fischer and Bachmann 1998). In short, genomic DNA of a common onion (cv. Kaba, accession number ALL917 from the Genebank of the IPK, Gatersleben, Germany) was cleaved with either *Rsa*I or *Hae*III and the resulting fragments ligated to adapters carrying an internal *Mlu*I recognition site. After hybridization with the pooled, biotinylated oligonucleotides $(CA)_{10}$, $(CAA)_8$ and $(GAA)_8$, microsatellite-containing DNA fragments were captured on streptavidin-coated magnetic beads (Dynal, Hamburg, Germany). Bound fragments were eluted, polymerase chain reaction (PCR)-amplified with an adapter-specific primer and subjected to a second enrichment cycle. The final PCR product was digested with *Mlu*I, cloned into the *BssH*II site of a modified pCRScript vector (Stratagene, La Jolla, Calif.), and used to transform chemically competent *E. coli* cells (Epicurian Coli® XL1-Blue MRF', Stratagene).

The plasmid clones with inserts were sequenced using the ThermoSequenase™ sequencing kit (Amersham Buchler GmbH & Co KG, Braunschweig, Germany). The clone sequences were aligned with Clustal X to detect possible duplicates. Primer pairs were designed in regions flanking the microsatellites with the software DesignerPCR™ 1.03 (Research Genetics, Huntsville, Ala.).

Plants

Plants were taken from the Gatersleben Genebank (accession numbers prefixed by ALL or K) and the collection of the Taxonomy Department (accession numbers prefixed by TAX) of the Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany (Table 1). A total of 83 *Allium cepa* L. accessions were studied. In addition, we analyzed 14 accessions of 6 species within section *Cepa* (Mill.) Prokh., *A. roylei* (accession number TAX5152), A. *vavilovii*, (TAX5238 and TAX5239), *A. cepa* of the cultivar group aggregatum (TAX3216), A. *cepa* of the 'common onion' group (TAX3731), *A. pskemense* (TAX1916), *A. praemixtum* (TAX5712), *A. oschaninii* (TAX1628, TAX2177 and TAX2528), A. *fistulosum* (TAX0430 and TAX0266), *A. altaicum*, (TAX5561 and TAX2760), 8 accessions of 5 species and one subspecies of the closely related section *Schoenoprasum* Dum., *A. schoenoprasum* ssp. *schoenoprasum* (TAX3465, TAX3744), *A. schoenoprasum* ssp. *latiorifolium* (TAX5432), *A. oliganthum* (TAX3201), *A. ledebourianum* (TAX3170), *A. altyncolicum* (K433), *A. karelinii* (TAX2592), *A. maximowiczi* (TAX2772), 2 species of section *Annuloprason* Egor., *A. fedschenkoanum* (TAX2560) and *A. atrosanguineum* (TAX2912), all within the subgenus *Rhizirideum* (G. Don ex Koch) Wendelbo (Hanelt *et al.* 1992).

DNA isolation and PCR analyses

DNA was isolated from leaves of onion plants or seedlings using the Nucleospin Plant Extraction Kit (Macherey & Nagel, Düren, Germany). The cultivar collection was analyzed at 15 microsatellite loci (Table 2). PCR was performed with the corresponding primer pairs, with one of the two primers 5' fluorescein-labeled using the following protocol: 32-µl sample contained 0.53 µ*M* of each primer, 0.22 m*M* dNTP, 0.75 U *Taq*-polymerase (Boehringer, Mannheim, Germany), about 30 ng template DNA in a buffer with 50 m*M* sodium salts and 2 m*M* magnesium ions. For rapid optimization of PCR conditions, touch-down thermo cycler programs were adopted where necessary. These start at or somewhat below the annealing temperature (T_A), calculated according to Breslauer *et al.* (1986), and then decrease for 1°C or 2°C in temperature after a certain number of cycles until a final temperature is reached, at which point the annealing steps of the remaining cycles are carried out. The three holds of these programs were 5 s at 94°C, 45 s at the cycle-dependent variable annealing temperature and 60 s at 72°C. PCR was always preceded by 2 min of pre-denaturation at 94°C and followed by 5 min post-synthesis at 72°C. The apparent annealing temperatures and numbers of cycles are given in Table 3 along with additional marker details. Markers without touch-down data have not been optimized this way and can be used with an apparent T_A of some degrees (°C) below the calculated T_A as given in the third column (Table 3).

PCR products were checked on 3% agarose gels (45 m*M* Tris-borate, 1 m*M* EDTA) and subsequently analyzed on an ABI 377 fluorescent sequencer (ABI, Weiterstadt, Germany) with a 12-cm apparent gel run distance, following the procedure recommended by the manufacturer. Resulting data were processed with GeneScan 3.1 and Genotyper 2.1 fragment analysis software (ABI). In addition, selected PCR products of *Allium* wild species and of *A. cepa* accessions that showed different polymorphic amplicons in the electrophoretic analyses were sequenced to show the origin of the amplified loci and the presence of microsatellite motifs. Bands of fragments from 50-µl or 100-µl reaction volumes were cut out of agarose gels, and the DNA was isolated using the NucleospinGelExtract Kit (Macherey & Nagel, Düren, Germany) to serve as templates in the sequencing reactions with the microsatellite primers. All sequencing reactions were performed using either BigDye™ (ABI) or ThermoSequenase™ (Amersham Buchler, Braunschweig, Germany) sequencing kit.

Results

Marker development

We have designed a set of 30 STMS primer pairs in flanking regions of onion sequences (*Allium cepa* L.) from the enriched library (Table 3). A multiple alignment of the 30 clone sequences with Clustal X revealed no duplicates (data not shown). However, 3 loci (AMS01, –20, –27) showed similar sequences, with one stretch of 18 matching bases in the 5' flanking region and extended similarities of 50–70% in the 3' flanking sequence, probably due to older locus duplications.

Of these 30 STMS 21 were thoroughly optimized in terms of PCR conditions and reproducibility and used in subsequent analyses. The core motifs of the markers included perfect microsatellites, imperfect and interrupted microsatellites of the perfect as well as the compound type (classification according to Jarne and Lagoda 1996). No correlation was found between the degree of polymorphism and a certain marker type.

Genotyping of onion accessions and landraces

Analysis of the landraces with four primer pairs (AMS08, AMS23, AMS25 and AMS26) yielded unique patterns of fragment sizes for the 83 genotypes even when information on allele sizes was not taken into account (Table 4). As a method for simplified genotyping

Table 1 Onion and shallot accessions and landraces. Accession numbers prefixed by A (which corresponds to ALL) and numbers prefixed by K are from the Genebank, and numbers prefixed by T (corresponds to TAX) originate from the living collection of the Taxonomy Department of the Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany; landraces without explicitly known names are shown with their collection number (CNr)

Number	Accession number	Name	Origin
1	K10099	CNr 12645	Albania
2	K10102	CNr 12674	Albania
3	K10105	CNr 12701	Albania
4	K09150	'bardhë e sukthit'	Albania
5	K09453	CNr 12267	Albania
6	K09630	CNr 12523	Albania
7	A0034	'Zitavska Obri'	CSFR
8	A0180	'Obrovska Zluta'	CSFR
9	K06309	'Alice'	CSFR
10	A0669	'Asenovgradska Kaba'	Bulgaria
11	A0963	'Sumperska'	CSFR
12	A0964	'Zazriva 2'	CSFR
13	A0965	'Moravska Polhora'	CSFR
14	K09703	'Vsetana'	CSFR
15	A1121	'Früka'	Germany
16	A0914	CNr 530	Georgia
17	K07091	'Ravalsviliani'	Georgia
18	K08238	CNr 2869	Georgia
19	A0548	CNr 120	Italy
20	A1437	'Cipolla Rossa di Lucca'	Italy
21	K09914	CNr 1277'	Italy
22	A0723	'nn' (K6980)	Canada
23	K07773	'nn'	Kazakhstan
24	A0734	CNr 115	Korea
25	A1415	CNr P852	Cuba
26	K07831	CNr P1870	Cuba
27	K08198	'Ajo Porro'	Cuba
28	K08191	ajo	Cuba
29	A0177	'Luctor'	Netherlands
30	A1211	NIZ (E 20473)	Netherlands
31	A1212	'Spirit F1'	Netherlands
32	A1215	'Santé'	Netherlands
33	A1216	'Pikant'	Netherlands
34	A0957	Yellow Shallot	Austria
35	A0960	Shallot (AUT 2)	Austria
36	A0920	'Stuttgart'	Romania
37	A1412	'Rosie de Turda'	Romania
38	A1421	'Caribe 71'	Romania
39	K9594	Celena Cibulea (Ukrainian Reddish Onion)	Romania
40	A0298	CNr 258	Slovakia
41	A0026	'Bessonovskij Mestnyj'	Soviet Union
42	A0155	'Pogarskij Mestnyj Ulucsennyj'	Soviet Union
43	A0161	'Novoselickij Mestnyj'	Soviet Union
44	A0304	'Strigunovskij Nosovskij'	Soviet Union
45	A0953	'Sibirskij Skorospelyj'	Soviet Union
46	A1218	Tschernuschka	Soviet Union
47	A0917	Kaba	Tadjikistan
48	K09179	CNr 32	Tunisia
49	A1209	Sweet Vidalea	USA
50	A0721	nn	USA
51	A0722	Yellow Potato	USA
52	A0731	Ruden	USA
53	A0732	Featherston	USA
54	A0733	Burkhart	USA
55	A1045	Frog's Legs Shallot	USA
56	T5552	nn	Vietnam
57	A0673	Primodore	United Kingdom
58	A0918	Hanka	(Unknown)
59	K09699	Jermor (A1351)	(Unknown)
60	K09696	Fuseor	Croatia
61	K10030	CNr 31 A	Croatia
62	K10047	CNr 98	Croatia
63	K10058	CNr 172	Croatia
64	K10082	CNr 271	Croatia
65	K10061	CNr 188	Croatia
66	K10089	CNr 340	Croatia
67	K09518	Red of Florence	Italy
68	K08607	Aviv Berlina	Israel
69	ALIS6 S	Alisa Craig	Germany
70	ZITT3 S	Zittauer Gelbe	Germany
71	STUR6 S	Stuttgarter Riesen	Germany
72	A0001	pear-shaped Yellow	Germany
73	A0027	Australian Brown	Australia
74	A0042	Zwaan's Great Winter	Netherlands
75	A0173	Produryin	Netherlands
76	A176 (K0761)	Produnos	Netherlands
77	A0178	Primodore (2)	Netherlands
78	A0306	Gurghiu	Romania
79	A0311	Calbenser Gerlinde	Germany
80	K09540	Cipolla Vernina di Firenze	Spain
81	K09573	Ceapa Alba de Gardine (=White Garden Onion)	Romania
82	K09565	nn	Portugal
83	A1327	Lemi	Finland
84	A0917	Kaba [SSR loci cloned from this genotype]	Tadzhikistan

Table 2 Loci used for analysis of the onion accession listed in Table 1. Number of alleles means the number of distinguishable sizes in the expected range, Non-homozygousity is the frequency of non-homozygous genotypes among the 83 accessions examined at the respective locus

Locus	Number of Alleles	Non-homozygosity
AMS04	6	0.131
AMS06	25	0.913
AMS07	8	0.785
AMS08	19	0.759
AMS10	16	0.796
AMS12	19	0.864
AMS13	18	0.906
AMS14	14	0.813
AMS16	19	0.898
AMS22	15	0.880
AMS23	26	0.906
AMS25	34	0.884
AMS26	20	0.891
AMS29	12	0.692
AMS30	23	0.887

in large data sets the rows of Table 4 are sorted by four-digit numbers composed from the counts of amplicon peaks found at the above four loci. Successive rows have been compared with their respective preceding rows. Therefore actual allele size comparison could be restricted to those accessions with equal peak count numbers.

Table 3 Microsatellites AMS01 to 30: core motifs and primer sequences. For details of the PCR protocols, see Materials and methods

STMS	Size[a] (bp)	T_A[b] (°C)	Annealing protocol[c]	Microsatllite motif	Forward and reverse primer (5' to 3')
AMS01	126	61.7	60°C 40-fold	(TGTA)5 (TG)9 GAAGAA	TCT TCC TAT AAT CTT CTC CTT TTG A TTC TAA CAC TTT TGT GCA CTC AA
AMS02	530	54.8	55°C 35-fold	CCACACCACACACACCACCA CACACCACA	GCA TTA ACT ATC TAA AAC ATT G CCA TCA ACT CAT AAC AGG T
AMS03	121	56.4	56°C 35-fold	(GT)21	TAA CCC TAG GAT GAG TTG AG GGA TTT CCT CTT GAG ATG A
AMS04	204	57.2	56°-54°-52°-50°-52°C 05–03–02–02–36 fold	(GTTTT)3 CTCTT(CT)3 (TTC)4 TC(TTC)2 (TC)2 TTCTTTTC TTTCTCT	TAT GTT TTC AGC TGC GAT GTG AG AAA TCT AAG CAC GGA TAC CAA GTG
AMS05	229	57.5	56°C 35-fold	(AT)9 (GT)18 (CT)3	TTG AAA TAG TGA GTT AAG CAT G ACG TGA ATT ATG AAG TGG AG
AMS06	147	62.8	67°–65°–63°–62°C 08–03–03–35-fold	(TA)3 TG (TA)3 (CA)18 (TA)2	GGT GCA TAG GGT CTC ATC TG ATT GAT TGT TTG TTT GGA TGT G
AMS07	114/174	68.0	67°–65°–63°–62°C 08–03–03–35-fold	GTTTCTGTTT (CTT)6 (TC)2 TTT(CTT)2	TGC GAA TGT GAG GTT TTC TGC CGA CCC GGA AAT TTC GAT C
AMS08	205	66.5	58°–56°–54°–53°–55°C 08–05–03–02–35-fold	(CTT)3 T (CTT)14 TT (CT)2 TCT	GCC ACG ATG TTG AGA TTT CG CCC GAA TAT CCC ACC AGT TC
AMS09	278	55.2	54°C 40-fold	(AT)9 (GT)19	ACA ACT TTC AAT TGC ATT C CGT GGA CTA ACT TAC TAT CTA TC
AMS10	157	60.0	58°–56°–54°–52°-54°C 05–05–04–02–35-fold	(AT)4 (GT)16	TTC ATG TTG TAT TGA GAT TTG G GAA GGA ATG GAA GCA GTT C
AMS11	92	61.2	62°–61°–60°–58°C 06–06–04–40-fold	(TC)23	CGA CGA ACC AAT ACC CTA TC TGG ATA GGG GTA GAA TTC AAG
AMS12	274	60.7	64°–62°–60°–58°–60°C 05–03–03–02—35-fold	(CA)25	AAT GTT GCT TTC TTT AGA TGT TG TGC AAA ATT ACA AGC AAA CTG
AMS13	168	61.2	64°–62°–60°–58°–60°C 04–03–03–02–35-fold	(GT)27 (AT)2	ACC TTT TAA ATT GAC GAT ATT CC CTG CAC TAT TCT GTG ATG TAT TTC
AMS14	169	62.1	60°–58°–56°C 03–03—35-fold	(CA)28 (TA)4	CCC CTG AGT AAA TTC AAA ATC C TCC TTA GTA TAA TTT CGG GGT AAC
AMS15	229	66.7	60°C 40-fold	(GA)24	ACC CCG AAC CAC GTA AAC C CCG ATT TTC CTT GCA TTC G
AMS16	261	63.2	62°–60°–58°–56°–58°C 08–05–03–02–35-fold	(CA)20 (TA)2	CTG CAT TAA AAC AAC CAA ACT TG GAG CTC CAC TTC TTC CAA ACT AG
AMS17	264	60.6	58°C 40-fold	(CA)7 TG (CA)21 (TA)3	AGT GGA CTC AAG GCA GAT G ATC ACC ATT CAC CGT TTA CT
AMS18	195	61.3	56°–55°–54°–53°–54°C 03–03–03–03–36-fold	(CA)20 (TA)3	ACT CGG GT GTT ATT CCA T CCA ATC AGA CAT ACC ATA CAA TC
AMS19	131	61.9	56°–55°–54°–53°–54°C 06–04–03–02–40-fold	GAAAAGAAGAAAGAGAAA (GAA)5 ACAGAA	GCT CTG ATA CCA AAT GTA ACG A CGA ATG TGA GGT TTT CTG C
AMS20	372	63.3	58°–56°–55°–54°–56°C 05–03–03–02–36-fold	(GT)24	TTG AGC AGC AGA ACC AGA C ATT CGG ACG CAA CAC ATC
AMS21	264	62.2	59°–58°–56°C 06–05–40-fold	(CA)25	GGT TGT TTC CAC TAC ACT TGA G CGT CCT TGG TAT TCT TGT GC
AMS22	310	63.0	64°–62°–61°–60°–59°C 03–03–03–02–35-fold	(TG)21	CAC CGT TTC CAT AAT CAA GG ATT TTT TGG GCA TTG TTG G
AMS23	157	62.5	60°–59°–58°–57°–58°C 08–05–03–03–35-fold	(AT)5 (GT)19	GCT GTT CAC TGG TCT ATC TGG ATT CGG TGC TGA TTT TCG
AMS24	161	60.8	56°–54°–52°–50°–53°C 06–04–03–03–38-fold	(TA)3 (CATA)7 ATA (CA)5 A (CA)15	GCT AAG TAG AAA CTA AGC GAT TGT TCA AAA ACA CCA AGC ACA TT
AMS25	235	65.3	65°–63°—61°–59°C 08–05–03–40-fold	(AC)21 (AT)3	GAG GGC AGT GTT AGC ATT CC GCA ACC TTT CCC CGA GAG
AMS26	213	52.6	56°–54°–52°–50°–52 °C 06–03–03–02–36 fold	(A)5 (CA)16	ATC TAA TCA AAG CAT AGT TG TTG TCC AAG TAG TTG TGA
AMS27	318	54.8	55°–54°–53°–52°–54°C 08–04–02–02–40-fold	(AT)7 (GT)25	TCC ACG AAT GAT TAC AAC ACA ACG CAA AAG TTC TTA
AMS28	250	56.7	55°C 40-fold	(AT)2 (AC)23	GTT GTC CTT TGC GTT TAC ATG GTT TCA TCA ATG TCC

Table 3 (continued)

STMS	Size[a] (bp)	T_A[b] (°C)	Annealing protocol[c]	Microsatllite motif	Forward and reverse primer (5' to 3')
AMS29	310	60.4	56°–55°–54°–53°–55°C 06–03–03–02–36-fold	AAG $(AAAG)_2$ GGATA $(GAA)_3$ AAGAAAGAGAAGAAAGAA $(CAA)_2$ $(CA)_2$	CAT CAG AAA ATC GCA TCA C TTG AAA CTT GGA AGG TTG TC
AMS30	342	60.2	57°–55°–53°–55°C 08–05–03–35-fold	$(CA)_8$ CG $(CA)_{22}$ $(TA)_4$	CAC TAA TGG GGT AAA TAA TGT TCT AC TTG CCT TGA AAT CCA GAC

[a] Two allele sizes are given when different attempts for primer design were undertaken at the respective locus
[b] T_A, Mean annealing temperature of the two primer oligonucleotides according to Breslauer et al. (1986). In most cases, 'touchdown' thermal cycler programs were used
[c] Annealing steps were carried out at temperatures separated by hypehens; these temperatures were maintained for the number of cycle counts beneath the respective annealing temperatures

Grouping of the accessions

Intraspecific analysis of the 83 genotypes at 15 microsatellite loci was conducted, and genetic distances were (calculated from *D1*, average squared differences (D_1) in repeat numbers for any two alleles, each one from a different population, with the latter in our case being represented by the 83 accessions (Goldstein *et al.* 1995). Genetic distances were directly computed from the resulting allele size data using freely available software (MICROSAT for DOS, provided by Eric Minch, Stanford University). Neighbor-joining tree data were calculated from distances using PHYLIP (compiled by Joseph Felsenstein, Washington University, algorithms according to Saitou and Nei 1987), and a dendrogram was plotted with TREEVIEW (by Roderic D.M. Page). This tree was fairly well resolved, and the groups partly reflect the geographical origins of landraces. One group was especially very well supported and contained most of the taxa from two tropical origins and all Italian and Austrian accessions with a bias towards subtype *aggregatum* (shallots and potato onions). A rather small group could be distinguished to combine Czech and Albanian onions on the basis of similarities in bulb morphology (Fig. 1).

Subgenus *Rhizirideum*

To check the capabilities of the markers outside their species of origin we analyzed 24 species of the sections *Cepa*, *Schoenoprasum* and *Annuloprason* within the subgenus *Rhizirideum*. Six of the 21 microsatellite primer pairs 6, AMS04, –06, –12, –22, –23 and –26, amplified in a somewhat broader range of species beyond *Allium cepa* L. Nevertheless, non-homoplastic polymorphism was observed only among the species close to *A. cepa,* i.e. *A. vavilovii*, *A. roylei, A. praemixtum*, *A. oschaninii*, *A. pskemense* and, in part, *A. altaicum* and *A. fistulosum* for which the obtained data were less consistent. Particularly in section *Schoenoprasum* very few samples exhibited alleles in the molecular weight range of *A. cepa*. One remarkable exception was discovered at AMS04 which showed almost no variability in onion (97% of 83 accessions carrying 1 of 6 alleles), whereas 10 informative alleles were observed in the 24 wild species studied.

We merged recent RAPD, internal transcribed spacer (ITS) and morphological analyses of the above taxa (Friesen and Blattner 2000; Friesen *et al.* 1999; Hanelt *et al.* 1992) and compared our STMS fragment size data to the resulting phenograms. This standard of comparison allows us to differentiate missing data from real null-alleles by interpreting our allele sizes in the context of expected results according to the relatedness we infer from ITS and RAPD study. If 1 species in section *Annuloprason* (e.g. *A. atrosanguineum* at AMS12) together with several members of section *Schoenoprasum* (*A. karelinii, A. maximovicii*, etc.) lack an allele at a given locus, we would expect null-alleles in contrast to when there are missing amplicons in closely related taxa (*A. roylei* at AMS12,) whereas more distantly related species carry the respective size allele (TAX3216 *A. oschaninii*).

We restrict the following description to some typical examples and refer to Fig. 3 for further details.

Exceptional alleles were found in two genotypes of partial unclear ancestry: *A. oschaninii* (TAX3216, 'gray shallot') and *A. schoenoprasum* ssp. *latiorifolium* (TAX5432), both of which are presumed to be old interspecific hybrids of still unresolved parentage (Friesen and Blattner 1999).

The occurrence of additional loci must be inferred from the presence of a third fragment in diploid plants *A. roylei*, *A. vavilovii* and *A. altaicum* at AMS06, AMS22 and AMS26, respectively. In species closest to *A. cepa*, the alleles of AMS22 are restricted to a small size range (306–316 bp) indicating 1 fixed main locus. In our preliminary screening, most of the species fit into the reference dendrogram. The putative allotetraploid *A. schoenoprasum* ssp. *latiorifolium* (TAX5432) carried 2 alleles in the size range observed in *A. cepa* L. relatives (306 and 316 bp) and 1 in the range typical for section *Schoenoprasum* (280 bp), indicating its genome originated from both onion and chives. Overall, the distribution of alleles of these loci agreed with but did not significantly add to results based on RAPD, ITS and morphological data. However, AMS04 differentiated *A. vavilovii* from *A. cepa,*

Table 4 Amplicon peaks of the 83 accessions from Table 1. The figures below the 4 markers of those alleles amplified using primer pairs AMS08, -23, -25 and –26. The size of the originally cloned allele size is given between parantheses

Accession	AMS08 (205 bp)				AMS23 (157 bp)				AMS25 (235 bp)				AMS26 (213 bp)			
A0001	205				183				207	211	213	219	209	219		
A0026	190				167	185			201	211	215		207	215		
A0027	187	220			141	183			191	201	203	217	213	225		
A0034	190	196			171				189				207	219		
A0042					137	181			201	211	213	221	217			
A0155	190				185				201	273			207	211	213	
A0161	190	193			145	167	175	185	187	201			207	217	219	231
A0173	166	267			149	167	181		201	213	247		213			
A0176	208				181				191	199			213			
A0177	193	208			173	185			201	207	211	221	213	219	221	
A0178	208				173	181			201	211	213		209	213		
A0180	190				173				187				207	211	217	
A0298	190				171	185			167	181	201		207	209	211	
A0304	187	205			183				201	227	271		211	215	219	227
A0306					143	163	181		201	211	213		213			
A0311	190				151	189			201	205	213		211			
A0548	157	184	190	214	145	171	185		201				209	219		
A0669	205	214			143				187				213			
A0673	190	214			139	185			181	187	201	247	211	219		
A0721	214				167	193			201	211	213		207	211	223	225
A0722	190	208			141	183			203	211	213	217	211	217		
A0723	190	208			171				143	201	207	211				
A0731	190	208			171	183			181	201	229		159	211	217	
A0732	190				145	171	183		137	139	273					
A0733	190	208			167	183			229	273	275	287	209	215		
A0734	190	214			145	167	185		201	211			207	223		
A0914	190				171				201				207	211	215	
A0918	205				179	183			207	255	267	301	213	219		
A0920	190	205			185				201	211	221		209	215	231	
A0953	190	208			171	185			201	249			211	217		
A0957	208	214			141	171			143	201	211	213	209	213	217	219
A0960	208				171				201	205	211	213	211	217		
A0963	208	215			141	171			189				207	213	217	
A0964	190				171				201	211	219		211	219	237	321
A0965	190				145	167			201	211			207	211	215	
A1045	214				171	183			185	187	189	271	207	209	215	219
A1121	190	193			139	175			203	211			209	211	219	
A1209	190				175	185			201	207	211	213	213	249		
A1211	190	208			183				219				207	211	221	
A1212	190	208			185				201	211			209	211	215	
A1215	190	211			183				201	217			207	209	211	223
A1216	190				135	169			201				207	209	223	
A1218	187	193			139	183			203	211	213		205	209	213	
A1327	154				171	187			201	211	213		207	213	219	
A1412	190				169	171			201	203	211	213	211	217	229	
A1415	190	205			175				201	211	213		211	213		
A1421	205	208			149	183			199	209	211	215	209	219		
A1437	190				143	171			201				205	211		
AK917	190	205			157	183			237				209	215	219	
ALIS6 S	190				173	179	183		167	201	271		213	221		
K06309	190	193	214		173				189	201						
K07091	190	214			141	169			203	217			213	217		
K07773	190				167				201	211	213		207	211	215	219
K07831	190	229			183				191	197	203	205	223	225	229	
K08198	190	193	205		187				171	189			239			
K08199	193	223			185				189				229	231		
K08238	190				145	185			201	207	211	213	209	217	219	
K08607	190	211			183				271				207	215	225	
K09150	190	223			185				189	201			207	209		
K09179	190	208			153	183			203	211	213		195	211	213	
K09453	190				185				189				207			
K09518	190	202			141	183			185	187			211	221		
K09540	190				137	171	183		201	211			211			
K09565	157	190			183				211	213			211	215		
K09573	190	247			103	183			201	205	213	219	211	217		
K09630	190	223			185				187				207	213		
K09696	190				145	181			155	167	181		207	211	213	

Table 4 (continued)

Accession	AMS08 (205 bp)			AMS23 (157 bp)				AMS25 (235 bp)				AMS26 (213 bp)			
K09699	190			145	183			201	205	213	247	207	211	217	
K09703	190	208		173				201	207			209	213	221	
K09914	190			173				201	207	211	213	209	215		
K10030	190	193		183				201	211	213	247	205	211	215	
K10047	187	190	208	129	181			155	167	201	211	207	211	213	
K10058	190			183				201	211	213	247	211	217		
K10061	190			181				273				207	211	219	225
K10082	175	190		169	181			201	205	211		213	221		
K10089	205	208		167	183			181	273			205	213	217	
K10099	190	214	223	141				201	207			213	225		
K10102	190	223		173				201	211			207	211		
K10105	190	223		171				201	211			207	219		
K9594	190	208		151	147	173	185	203	211	213		211	215	217	
STUR6 S	190	193		141	181			201	207	213	255	211	213	219	
T5552	190	214		171	185			187	201	271	275	207	217	219	
ZITT3 S	190	211		167	173	181		201	271			207	213	221	225

Table 5 Twenty-four accessions of 16 *Allium* species representing the three sections *Cepa*, *Schoenoprasum* and *Annuloprasum* within the subgenus *Rhizirideum.* Plants were obtained either from the Genebank (prefix K) or from the Taxonomy Department (prefix TAX) of the Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany. Extracted total genomic DNA was analyzed with 6 microsatellites. Results are given in Fig. 3

Number	Section	Accession number	Species of the genus *Allium* L.
1	*Annulopraso*n	TAX2912	*atrosanguineum* Kar. *et* Kir.
2		TAX2560	*fedschenkoanum* Regel
3	*Schoenoprasum*	TAX3201	*oliganthum* Kar. et Kir.
4		TAX3170	*ledebourianum* Roem. *et* Schult.
5		K000433	*altyncolicum* Friesen
6		TAX2592	*karelinii* Poljak.
7		TAX2772	*maximowiczi* Regel
8		TAX3744	*schoenoprasum* L.
9		TAX3465	*schoenoprasum* L.
10		TAX5432	*schoenoprasum ssp. latiorifolium* S.R.M. *et al.*
11	*Cepa*	TAX0430	*fistulosum* L.
12		TAX0266	*fistulosum* L.
13		TAX5561	*altaicum* Pall.
14		TAX2760	*altaicum* Pall.
15		TAX1916	*pskemense* B. Fedt.
16		TAX2177	*oschaninii* O. Fedt.
17		TAX2528	*oschaninii* O. Fedt.
18		TAX1628	*oschaninii* O. Fedt.
19		TAX3216	*oschaninii* O. Fedt. (prev. *cepa*) 'gray shallot'
20		TAX5712	*praemixtum* Vved.
21		TAX5152	*roylei* Stearn
22		TAX5238	*vavilovii* M. Pop. et Vved.
23		TAX5239	*vavilovii* M. Pop. et Vved.
24		TAX3731	*cepa* L. (onion)

which were been grouped together on the basis of previous data. Similarly, *A. fistulosum* and *A. altaicum* could be separated with any one of primer pairs AMS04, AMS06, AMS12 or AMS22. The 'gray shallot' (*A. oschaninii*, accession number TAX3216) was separated from other *A. oschaninii* at all 6 loci and showed either alleles that were unique in the inner two branches of the reference dendrogram (at AMS04, –12, –23 and –26) or at least in its species (AMS06) or a null allele, whereas the remaining accessions of *A. oschaninii* amplified (AMS22).

Sequences of amplicons

Alleles were sequenced using the microsatellite primers at 3 loci, 2 amplified from onion accessions and 1 amplified from the *Allium* wild species given in Table 5. We confirmed the identity of polymorphisms detected by fluorescent fragment analysis as variable number of tandem repeats (VNTRs) at microsatellite loci with the expected core motif and flanking sequences of stable length and conserved sequences. From these results we inferred that our markers revealed size variation due to typical microsatellite slippage events in *Allium* wild species (Fig. 4) and onion (Figs. 5, 6).

Discussion

Almost the whole set of STMS markers were found to be ideally suited for genotyping. Three microsatellites showed 23 (Fig. 2), 26 and 34 allele sizes, respectively,

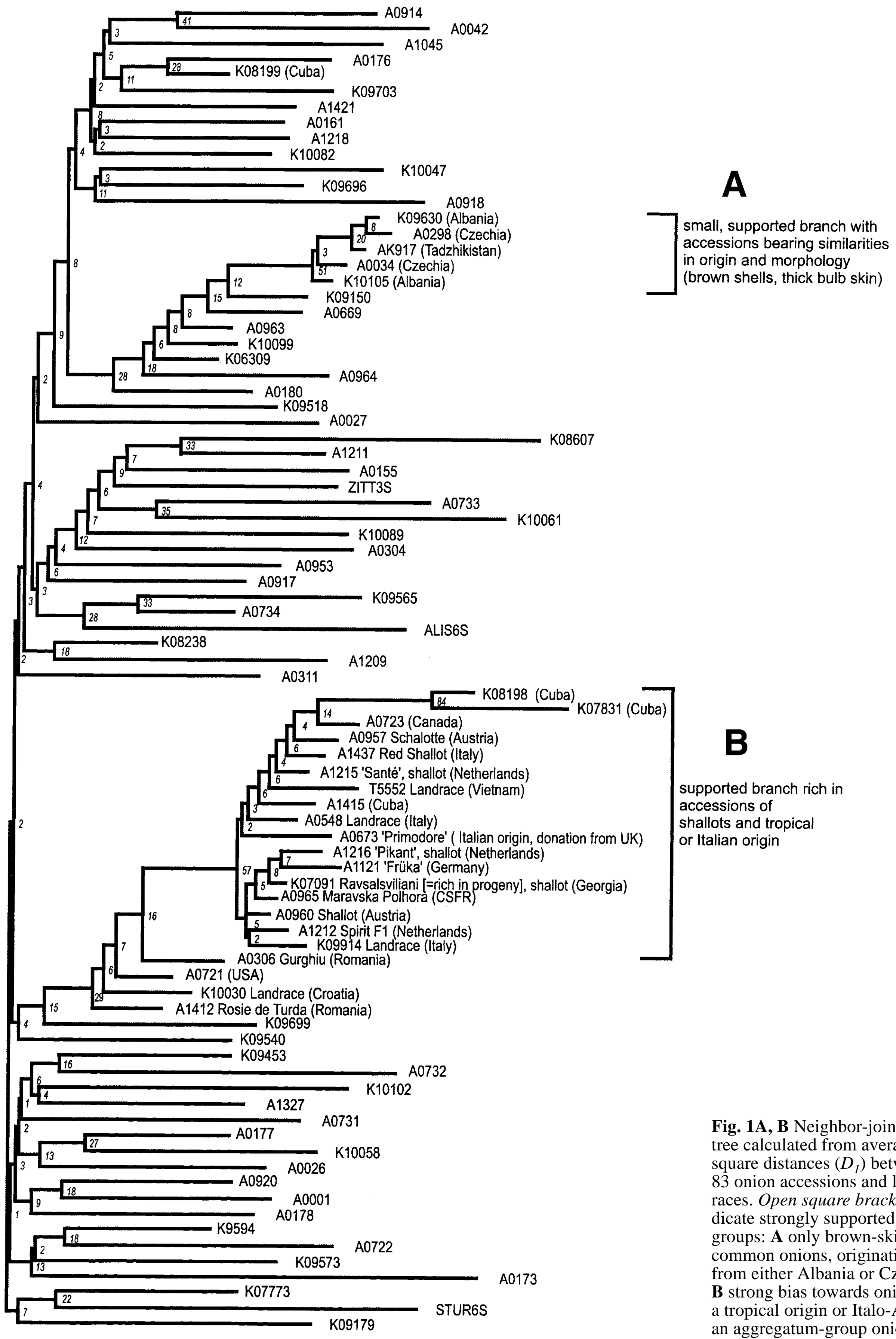

Fig. 1A, B Neighbor-joining tree calculated from average square distances (D_1) between 83 onion accessions and landraces. *Open square brackets* indicate strongly supported groups: **A** only brown-skinned common onions, originating from either Albania or Czechia, **B** strong bias towards onions of a tropical origin or Italo-Austrian aggregatum-group onions

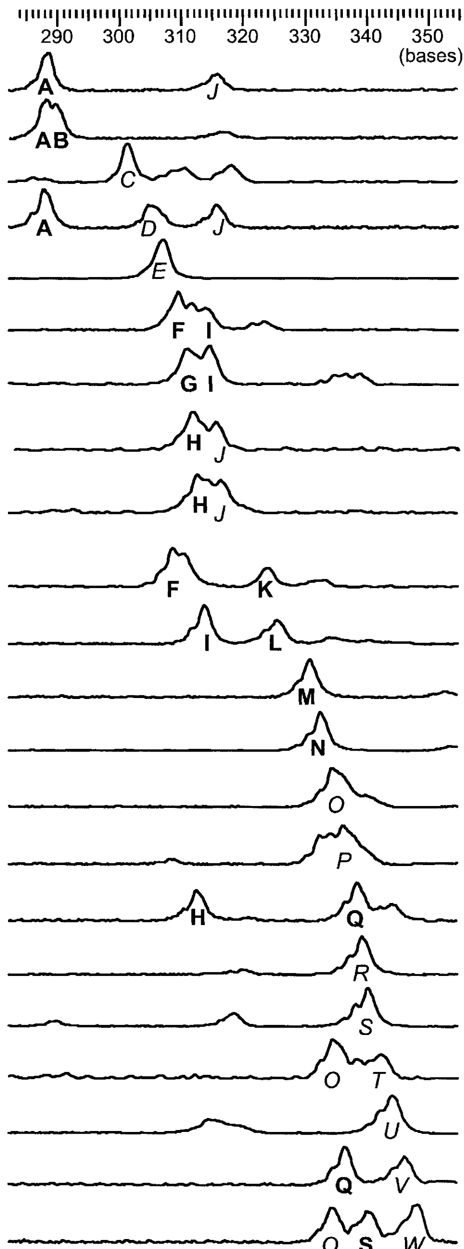

Fig. 2 Fluorogram plots of 23 size alleles (*A-W*) at the interrupted compound microsatellite locus AMS30. Alleles differ for multiples of two or three bases from the sequenced allele (341 bp). *A* and *B* may contain deletions in the flanking region, *C* through *W* can be explained by core repeat variation of the heterogeneous motifs. The microsatellite motif at AMS30 is GAAA$(GA)_2$ AGAAA$(GA)_2$ TT$(GAA)_5$ TAGAAA

in our accessions (Table 2), which is similar to the extraordinary polymorphism of microsatellites in other crops (e.g. Saghai-Maroof *et al.* 1994). This recommends them for use in germplasm identification, the legal protection of cultivars, for genetic resources conservation and breeding programs. However, the value of these microsatellites for the determination of intraspecific relatedness needs to be examined more closely. Microsatellite variation depends on a high mutation rate, and we should not expect stable association with slowly changing single or multigenic traits such as bulb shape, skin color, day-length response or other morphological variation. Since many widely cultivated onion accessions have been distributed rapidly by man over different parts of the globe, it should be possible in some cases to trace this distribution. This may be the case for the long branch of the dendrogram combining Italian and tropical shallots and onions (Fig. 1 B). Actually, onion germplasm was originally transferred from southern Italy via Spain to Middle America (C.M. Messiaen, personal communication). An interesting feature of this group is a bias towards shallots. This could be due to a neglected practice of transplanting and seedbed preparation in places where colonial settlers stuck to more profitable crops like coffee, sugar and tobacco, and onion cultivation was continued in small housegardens. This may have led to a preferred selection of rapidly multiplying bulbs. Also, we should consider the fact that European onions in tropical climates may neither bolt nor form large bulbs. This could lead to enhanced selection for shallot or potato onion traits such as poor or missing floral induction, no dormancy and, consequently, numerous small bulbs. Another strongly supported but rather small group consists of onion landraces obtained from Albanian and Czech sources and does not correspond to a documented association (Fig. 1 A).

Classification above the species level is usually beyond the capabilities of this highly mutable marker type. Homoplasy accumulates quickly at microsatellite sites, and there are cases of altered or entirely different flanking regions in more distant taxa (Röder *et al.* 1995). However, there are a few examples of the successful application of microsatellites in interspecific analyses (or even intergeneric crosses, Kijas *et al.* 1995). In *Allium* we could rely upon detailed taxonomical data and established analyses with other markers. Only a few microsatellites met the conditions for intraspecific analyses such as low polymorphism among groups of the species. A microsatellite approach to phylogenies in *Allium* species that are more distantly related to *A. cepa* should be restricted to species without null-alleles in closer relatives of *A. cepa*. This is the case in the section *Cepa*. However, as we extended analysis to more distant branches of the merged RAPD and ITS tree, more null-alleles occurred depending on the microsatellite used. Most of the homoplasy was expected at AMS23 and AMS12 which showed a high degree of polymorphism in intraspecific analyses. At AMS06 and AMS26 we found amplification signals throughout all species studied. With these primer pairs the detection of at least 1 additional locus hampers the assessment of possible homoplasy. The phylogeny derived from earlier analyses was supported or at least not contradicted in the first and second branch of section *Cepa*, including the species *A. cepa*/*A. vavilovii*/*A. roylei*, and *A. praemixtum, A. oschaninii*/*A. pskemense*. Some alleles of species *A. altyncolicum* and *A. pskemense* showed unexpected size similarities to the close relatives of *A. cepa* at (AMS04 and AMS23) and

Taxon	AMS-04	AMS-06	AMS-12	AMS-22	AMS-23	AMS-26
TAX2912 atrosa	249	-	-	-	-	183
TAX2560 fedsch	237 258	131	256	278 288	- (205)	199
TAX3201oligant	-	123 131135	-	270274 280	-	199
TAX3170 ledebo	-	129	262	270274 280	-	175 183 187
K0433 altyncolic	237	129 135	-	274	(123)	199 207
TAX2592 karelin	-	129 135 157	-	274 284	-	173 181 183
TAX2772 maxim	-	129	-	276	-	199 207 213
TAX3744 schoe	-	129	-	274	-	195 199
TAX3465 schoe	-	129	-	270 280	-	199
TAX5432 schoe (ssp. latiorifolium)	174	129	-	280 306316	-	199 227
TAX0430 fistulo	237	129 149	234242	-	-	195 221
TAX0266 fistulo	237	129 149	242	-	-	177 199 211
TAX5561altaic	-	123 129 149	258	260 282 288	-	199
TAX2760 altaic	-	123 129 149	264	260 282 288	-	199 211
TAX1916 pskem	237 255	129 135	-	270	157	183 195 199
TAX2177 oscha	252	129 147	234	270	-	201
TAX2528 oscha	252	129 135	234	270	-	201
TAX1628 oscha	252	129 147	234	274	-	201
TAX3216 oscha (grey shallot)	219	123 129	268	-	137 157	201 207
TAX5712 praem	237 255	129 135	234	270 310	-	195 199
TAX5152 roylei	-	123 129 155	-	270 280	-	201 209 213
TAX5238 vavilo	237	129 157	268	272 280 310	139 145	201
TAX5239 vavilo	243	129 157	268	280 310	141145	201
TAX3731 cepa	240	129 157	268 278	310	145 161	201 209 213

Dendrogram section labels: An, Sh, Ce

Fig. 3 Size alleles of 24 species of *Allium* subgenus *Rhizirideum* at 6 microsatellite loci. A reference dendrogram reflecting recent data from other markers such as RAPDs, ITSs and morphology is given on the *left* together with accession numbers from Table 1 and the appropriate species names. ● Branch nodes leading to the sections, An *Annuloprason*, Sh *Schoenoprasum*, Ce *Cepa*

Fig. 4 Two aligned sequences of AMS04: the sequence cloned from *A. cepa* (ALL917) and the amplicon of *A. vavilovi* (TAX5239). Dotted box Microsatellite sites. (*bold framed box*) primer primer binding sites, – deletions caused by microsatellite length variation; + absent residues due to sequencing procedure

```
A. vavilovii   ++++++++++++++++++++++ ++++++CAATTTCTGTTTTGTAAAGATCAT
A. cepa        GGACTAACTCTAATTGATGAATG TATATACAATTTCTGTTTTGTAAAGATCAT

A. vavilovii   CAATACGATATTATATGTTTTCAGCTGCGATGTGAGGTTCTTTATT GTTCTGTT
A. cepa        CAATACGATATTATATGTTTTCAGCTGCGATGTGAGANTCTTTATT GTTTTGTT

A. vavilovii   TTGTTTTCTCTTCTCTCTTTCTTCTCTTCTCTTCTTCTCTCTTCTTCTTTTCT
A. cepa        TTGTTTTCTCTTCTCTCTTTCTTCTCTTCTCTTCTTCTCTCTTCTT---TTCT

A. vavilovii   TTCTCT TGACTTTCCTTCAGTCTGTGCTTTTTTGGGTCGGACATGTGTGGTTTAT
A. cepa        TTCTCT TGACTTTCCTTCAGTCTGTGCTTTTTTGGGTCGGACATGTGTGGTTTAT

A. vavilovii   TGGT CAGTTTAGAGGGTATGGATTT
A. cepa        TGGT CAGTTTAGAGGGTATGGATTT
```

those of *A. ledebourianum* at (AMS12), and *A. karelinii* showed common amplicon sizes with *A. ledebourianum* at AMS26, whereas the other members from section *Schoenoprasum* showed no alleles (AMS04, AMS23 in *A. altyncolicum*) or at least no alleles in the respective size range (AMS26). This may not reflect relatedness but rather be due to point mutations in primer sites in those species of the section *Schoenoprasum* which failed to amplifiy. The tetraploid *A. altyncolicum* has the largest known genome in this section, even larger than that of tetraploid chive (Ohri *et al.* 1998) This may account for the exceptional rise of homoplasy a at distant relationship, where null-alleles predominate.

The cultivated 'gray shallot' could be separated from the other *A. oschaninii.* But at different loci this cultivar showed size alleles resembling those found in *A. cepa* (AMS06, AMS12 and AMS23), *A. oschaninii* (AMS26, AMS06) and even *A. pskemense* (AMS23) or sizes which did not resemble expected relations. This may both question and form a new background to the analyses of Friesen and Klaas (1998), who classified the 'gray shallot' formerly included in *A. cepa,* as *A.*

Fig. 5 Cloned sequence of AMS16 and aligned amplicon sequences from 6 onion accessions (compare Table 1, nos. 84, 15, 12, 20, 1, 3 and 60). *Dotted box* Microsatellite sites, *bold framed boxes* primer binding sites: - deletions caused by microsatellite length variation; + absent residues due to sequencing procedure

```
AMS16_Clone    +++++++++++++GACTAACTAACATGACTTGTCTACACAATTTAAACAA CTGCATTAA
FRÜKA/GER      ++++++++++++++++++++++++++++++++++++++++++++++++++ CTGCATTAA
ZAZRIVA2/CSFR  ++++++++++++++++++++++++++++++++++++++++++++++++++ +++++++++
C. ROSSA/ITA   ++++++++++++++++++++++++++++++++++++++++++++++++++ +++++++++
LANDRACE1/ALB  ++++++++++++++++++++++++++++++++++++++++++++++++++ +++++++++
LANDRACE2/ALB  ++++++++++++++++++++++++++++++++++++++++++++++++++ +++++++++
LANDRACE/CRO   ++++++++++++++++++++++++++++++++++++++++++++++++++ +++++++++

AMS16 Clone    AACAACCAAACTTG ATGATCAAAGCATAATAAACATCCATGGCAAACTTATCACATCATC
FRÜKA/GER      AACAACCAAACTTG ATGATCAAAGCATAATAAACATCCATGGCAAACTTATCACATCATC
ZAZRIVA2/CSFR  ++++++++++++++ ++++++++++++++++++++++++++++++++++++++++++++TC
C. ROSSA/ITA   ++++++++++++++ ++++++++++++++++++++++++++++++++++++++++++++++
LANDRACE1/ALB  ++++++++++++++ +++++++++++++++++++++++++++++++++++++++CATTATC
LANDRACE2/ALB  ++++++++++++++ ++++++++++++++++++++++++++++++++CTTATCACATCATC
LANDRACE/CRO   ++++++++++++++ +++++++++++++++++++++++++++++++++++++++CATCATC

AMS16 Clone    ATTAT CACACACACACACACACACACACACACACACACACACATATA AAGTTTCGTAG
FRÜKA/GER      ATTAT CACACACACACACACACACACA----------------TATA AAGTTTCGTAG
ZAZRIVA2/CSFR  ATTAT CACACACACACACACACACACACACACACACACACA--TATA AAGTTTCGTAG
C. ROSSA/ITA   CTTAT CACACACACACACACACACACACACACACACACACA--TATA AAGTTTCGTAG
LANDRACE1/ALB  ATTAT CACACACACACACACACACACACACACACACA------TATA AAGTTTCGTAG
LANDRACE2/ALB  ATTAT CACACACACACACACACACACACACACACACA------TATA AAGTTTCGTAG
LANDRACE/CRO   ATTAT CACACACACACACACACACACAAACACACA--------TATA AAGTTTCGTAG
                **** ************************                **** ***********

AMS16_Clone    AGTTTTCTCACCTTTATTGGATACCAAACTTACGAAAACCGACTGTAGCTTCGTTACGAT
FRÜKA/GER      AGTTTTCTCACCTTTATTGGATACCAAACTTACGAAAACCGACTGTAGCTTCGTTACGAT
ZAZRIVA2/CSFR  AGTTTTCTCACCTTTATTGGATACCAAACTTACGAAAACCGACTGTAGCTTCGTTACGAT
C. ROSSA/ITA   AGTTTTCTCACCTTTATTGGATACCAAACTTACGAAAACCGACTGTAGCTTCGTTACGAT
LANDRACE1/ALB  AGTTTTCTCACCTTTATTGGATACCAAACTTACGAAAACCGACTGTAGCTTCGTTACGAT
LANDRACE2/ALB  AGTTTTCTCACCTTTATTGGATACCAAACTTACGAAAACCGACTGTAGCTTCGTTACGAT
LANDRACE/CRO   AGTTTTCTCACCTTTATTGGATACCAAACTTACGAAAACCGACTGTAGCTTCGTTACGAT
               ************************************************************

AMS16_Clone    CGAATCAAAGGCTACGAAATCAAGCATTGGACCTTTGAATCGCCCTGGC CTAGTTTGGAA
FRÜKA/GER      CGAATCAAAGGCTACGAAATCAAGCATTGGACCTTTGAATCGCCTTGGC +++++++++++
ZAZRIVA2/CSFR  CGAATCAAAGGCTACGAAATCAAGCATTGGACCTTTGAATCGCCCTGGC +++++++++++
C. ROSSA/ITA   CGAATCAAAGGCTACGAAATCAAGCATTGGACCTTTGAATCGCCTTGGC +++++++++++
LANDRACE1/ALB  CGAATCAAAGGCTACGAAATCAAGCATTGGACCTTTGAATCGCCTTGG+ +++++++++++
LANDRACE2/ALB  CGAATCAAAGGCTACGAAATCAAGCATTGGACCTTTGAATCGCCTTGGC +++++++++++
LANDRACE/CRO   CGAATCAAAGGCTACGAAATCAAGCATTGGACCTTTGAATCGCCTTGGC +++++++++++
               ******************************************** ***

AMS16_Clone    GAAGTGGAGCTC TCAATCAATGGTGATGGAGGTTGTGGCTGAGAGTTTGGAAGGTGGAGA
FRÜKA/GER      ++++++++++++ ++++++++++++++++++++++++++++++++++++++++++++++++
ZAZRIVA2/CSFR  ++++++++++++ ++++++++++++++++++++++++++++++++++++++++++++++++
C. ROSSA/ITA   ++++++++++++ ++++++++++++++++++++++++++++++++++++++++++++++++
LANDRACE1/ALB  ++++++++++++ ++++++++++++++++++++++++++++++++++++++++++++++++
LANDRACE2/ALB  ++++++++++++ ++++++++++++++++++++++++++++++++++++++++++++++++
LANDRACE/CRO   ++++++++++++ ++++++++++++++++++++++++++++++++++++++++++++++++
```

```
Or  GCCACGATGTTGAGATTTCG TAATTGTTTTAGTTTCTTCTATTTTGT CTTCTTCTTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT------TT
Co  ++++++++++++++++++++ +++++GTTTAGTTTCTTCTATTTTGT CTTCTTCTTTCTTCTTCTTCTTCTTCTTCTT------------------------TT
Ag  ++++++++++++++++++++ ++++++++++AGTTTCTTCTATTTTGT CTTCTTCTTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTTT
                         ******************************************************

Or  CTCTTCT ACTCCCTTCTTTATTTCCCATAAAGATCAGGTTTTCCATTTAGTTTGGACGGGACATGTGTGGTTTGTTGTTAGTTT GAACTGGTGGGATATTCGGG CCT
Co  CTCTTCT ACTCCCTTCTTTATTTCCCATAAAGATCAGGTTTTCCATTTAGTTTGGACGGGACATGTGTGGTTTGTTGTTAGTTT GAACTGGTGGGATATTCGGG CCT
Ag  CTCTTCT ACTCCCTTCTTTCTTTCCCATAAAGATCAGGTTTTCCATTTAGTTTGGACGGGACATGTGTGGTTTATTGTTAGTTT GAACTGGTGGGATATTCGGG CCT
    ******************** ***************************************************** *******************************
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Fig. 6 Three aligned sequences of AMS08: originally cloned STMS (*Or*: ALL917 Kaba, Table 1, no. 84), amplicon of a common onion (*Co:* ALL1209 Sweet Vidalea®) and amplicon of a shallot (*Ag:* TAX 1810). Dotted box Microsatellite sites. (*bold framed box*) primer primer binding sites, (–) deletions caused by microsatellite length variation; (+) absent residues due to sequencing procedure

oschaninii based on genome in situ hybridization (GISH) results.

Allium altaicum and *A. fistulosum* were separated at all microsatellite loci, except for AMS12. The ancestry of the cultivated *A. fistulosum* from *A. altaicum* (Friesen *et al.* 1999) was not contradicted by any of the microsatellite markers but supported to a certain extent by AMS06 and AMS22. Also the results at the loci AMS04 and AMS12 suggest this evolutionary direction, since the common size allele in either *A. fistulosum* accession supports a monophyletic origin. Friesen *et al.* (1999) grouped the *A. altaicum* accessions TAX5561 separately from TAX2760 based on RAPD markers and, in a different context, on PCR-RFLP. This differentiation was supported by AMS12 and AMS26. The closely related *A. roylei*, *A. vavilovii* and *A. cepa* could be easily distinguished by the size alleles of single primer pairs AMS04,

–12, –22, –23, and –26. Finally, the microsatellites proved to be valuable markers for genotyping purposes and for shedding light on interspecific relationships in the section *Cepa*.

Acknowledgments We thank the European Commission for funding (FAIR Project CT 95–465) and Mrs. Petra Oswald for expert technical assistance.

References

Bark OH, Havey MJ (1995) Similarities and relationships among open-pollinated populations of the bulb onion as estimated by nuclear RFLPs. Theor Appl Genet 90:607–614

Barnes SR, James AM, Jamieson G (1988) The organization, nucleotide sequence and chromosomal distribution of a satellite DNA from *Allium cep*a. Chromosoma 92:185–192

Bennet MD, Smith JB (1991) Nuclear DNA amounts in angiosperms. Philos Trans R Soc London (Ser B) 334:309–345

Blair MW, McCouch SR (1997) Microsatellite and sequence-tagged site markers diagnostic for the rice bacterial leaf blight resistance gene *xa-5*. Theor Appl Genet 95:174–184

Bradeen JM, Havey MJ (1995) Randomly amplified polymorphic DNA in bulb onion and its use to assess inbred integrity. J Am Soc Hortic Sci 120:752–758

Breslauer KJ, Frank R, Blöcker H, Marky LA (1986) Predicting DNA duplex stability from the base sequence. Proc Natl Acad Sci USA 83:3746–3750

Fahima T, Röder MS, Grama A, Nevo E (1998) Microsatellite DNA polymorhpism divergence in *Triticum dicoccoides* accessions highly resistant to yellow rust. Theor Appl Genet 96:187–195

Fischer D, Bachmann K (1998) Microsatellite enrichment in organism with large genomes (*Allium cepa* L.). Biotechniques 24:796–802 24:796–802

Friesen N, Blattner FR (2000) RAPD analysis reveals geographic differentiations within *Allium schoenoprasum* L. (*Alliaceae*). Plant Biol (in press)

Friesen N, Klaas M (1998) Origin of some minor vegetatively propagated *Allium* crops studied with RAPD and GISH. Genet Res Crop Evol 45:511–523

Friesen N, Pollner S, Bachmann K, Blattner FR (1999) RAPDs and noncoding chloroplast DNA reveal a single origin of the cultivated *Allium fistulosum* from *A. altaicum* (*Alliaceae*). Am J of Bot 86:554–562

Geistlinger J, Weising K, Kaiser WJ, Kahl G (1997) Allelic variation at a hypervariable compound microsatellite locus in the ascomycete *Ascochyta rabiei*. Mol Gen Genet 256:258–305

Goldstein DB, Riuz Linares A, Cavalli-Sforza LL, Feldman MW (1995) An evaluation of genetic distances for use with microsatellite loci. Genetics 139:463–671

Hanelt P, Schultze-Motel J, Fritsch R, Kruse J, Maass HI, Ohle H, Pistrick K (1992) Infrageneric grouping of *Allium* – the Gatersleben approach.. In: Hanelt P, Hammer K, Knüpffer H (eds.). The Genus *Allium* – taxonomic problems and genetic resources. IPK, Gatersleben, Germany, pp 107–123

Jarne P, Lagoda PJL (1996) Microsatellites, from molecules to populations and back. Trends Ecol Evol 11:424–429

Jones CJ, Edwards KJ, Castaglione S, Winfield MO, Sala F, Wiel C van de, Bredemeijer G, Vosman B, Matthes M, Daly A, Brettschneider R, Bettini P, Buiatti M, Maestri E, Malcevschi A, Marmiroli N, Aert R, Volckaert G, Rueda J, Linacero R, Vazquez A, Karp A (1997) Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Mol Breed 3:381–390

Kijas JMH, Fowler JCS, Thomas MR (1995) An evaluation of sequence tagged microsatellite markers for genetic analysis within *Citrus* and related species. Genome 38:349–355

Klaas M (1998) Applications and impact of molekular markers on evolutionary and diversity studies in the genus Allium. Plant Breed 117:297–308

Korzun V, Röder MS, Ganal MW, Worland AJ, Law CN (1998) Genetic analysis of the dwarfing gene (*Rht8*) in wheat. Part I. Molecular mapping of *Rht8* on the short arm of shromosome 2D of bread wheat (*Triticum aestivum* L.) Theor Appl Genet 96:1104–1109

Maughan PJ, Saghai-Maroof MA, Buss GR(1995) Microsatellite and amplified sequence in cultivated and wild soybean. Genome 38:715–723

Moran, PAP (1975) Wandering distributions and the electrophoretic profile. Theor Pop Biol 8:318–330

Mudge PB, Cregan PB, Kenworthy JP, Kenworthy WJ, Orf JH, Young ND (1997) Two microsatellite markers that flank the major soybean cyst nematode resistance locus. Crop Sci 37: 1611–1615

Neu C, Kaemmer D, Kahl G, Fischer D, Weising K (1999) Polymorphic microsatellite markers for the banana pathogen *Mycosphaerella fijiensis*. Mol Ecol 8:513–525

Ohri D, Fritsch RM and Hanelt P (1998) Evolution of genome size in *Allium* (*Alliaceae*). Plant Syst Evol 210:57–86

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed 2:225–238

Röder MS, Plaschke J, König SU, Börner A, Sorrells ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246:327–333

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Saghai-Maroof MA, Biyashev RM, Yang GP, Zhang Q, Allards RW (1994) Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. Proc Natl Acad Sci USA 91:5466–5470

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. J Mol Evol 4:406–425

Sharon D, Adato A, Mhameed S, Lavi U, Hillel J, Gomolka M, Epplen C, Epplen JT (1995) DNA fingerprints in plants using simple sequence repeat and minisatellite probes. Hortic Sci 30:109–112

Smulders MJM, Bredemeijer G, Rus-Kortekaas W, Arens P, Vosman B (1997) Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersicon* species. Theor Appl Genet 97:264–272

Stack SM, Comings DE (1979) The chromosomes and DNA of *Allium cepa*. Chromosoma 70:161–181

Taramino G, Tingey S (1996) Simple sequence repeats for germplasm analysis and mapping in maize. Genome 39: 277–287

Weising K, Fung RWM, Keeling DJ, Atkinson RG, Gardner RC (1996) Characterisation of microsatellites from *Actinidia chinensis*. Mol Breed 2:117–131

Weising K, Winter P, Hüttel B, Kahl G (1998) Microsatellite markers for molecular breeding. J Crop Prod 1:113–143

Wilkie SE, Isaac PG, Slater RJ (1993) Random amplified polymorphic DNA (RAPD) markers for genetic analysis in *Allium*. Theor Appl Genet 86:497–504

Xiao J, Li J, Yuan L, McCouch SR (1996) Genetic diversity and its relationships to hybrid performance and heterosis in rice as revealed by PCR-based markers. Theor Appl Genet 92:637–643