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Random amplified polymorphic DNA (RAPD) markers for genetic analysis in *Allium*

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Abstract. RAPD analysis was applied to onion (*Allium cepa*) and other *Allium* species in order to assess the degree of polymorphism within the genus and to investigate if this approach was suitable for genetic studies of onion. Seven cultivars of *A. cepa*, including shallot, and single cultivars of Japanese bunching onion (*A. fistulosum*), chive (*A. schoenoprasum*), leek (*A. ampeloprasum*), and a wild relative of onion (*A. roylei*), were evaluated for variability using a set of 20 random 10-mer primers. Seven out of the twenty primers revealed scorable polymorphisms between cultivars of *A. cepa* and these will be further evaluated for use in genetic mapping. Wide variations in banding profiles between species were observed with nearly every primer tested. These were assessed for use in systematic studies within the genus. Ninety-one band positions were scored (+/−) for all the cultivars studied. Genetic distances between each of the cultivars were calculated and cluster analysis was used to generate a dendrogram showing phylogenetic relationships between them. The resulting analysis was in broad agreement with previous classifications of the species studied, confirming the validity of the method. However, amongst the species studied, it placed *A. roylei* as the closest relative of *A. cepa*, questioning the current classification of the former species in the section Rhizideum.

Key words: Onion – Random primer PCR – Phylogenetic analysis – Genetic distance

Introduction

Allium is a large genus of approximately 600 species and contains several major agricultural crops including the bulb onion (*Allium cepa*), shallot (*A. cepa* syn. *A. ascalonicum*), Japanese bunching or Welsh onion (*A. fistulosum*), chive (*A. schoenoprasum*), garlic (*A. sativum*) and leek (*A. ampeloprasum* syn. *A. porrum*). All the common domesticated *Alliums* have a basic chromosome number of 8 and most are diploids (*A. cepa*: 2n=2x=16) (McCollum 1976).

Classification of such a large genus has proved difficult and many ambiguities still remain (Hanelt 1990). Vvedensky (1944) classified the cultivated *Allium* species into four sections, Cepa (bulb onion), Phyllodolon (Japanese bunching onion), Porrum (garlic and leek) and Rhizirideum (chive). A later classification based on morphological criteria, crossability, and karyotype (Traub 1968), similarly divided them among four sections (Allium, Cepa, Fistulosa and Rhizirideum) with further divisions into sub-sections. However, difficulties arise because there are relatively few morphological characters upon which to base a classification system and because strong barriers to crossing separate even morphologically-similar species. Recently it has been suggested that there could be a role for genetic markers in systematic studies of the species of *Allium* (Havey 1991).

Despite the major position of onion as a vegetable crop, very little genetic information is available for it. To-date no single genes affecting either morphological characters or disease resistance have been identified and reports of chromosomal gene loci are limited to ribosomal genes (Riocroch et al. 1992) and 12 isozyme loci (Peffley et al. 1985; Peffley and Currah 1988; de Vries, personal communication). As a result of the paucity of available genetic markers, onion breeding still relies heavily on phenotypic selection methods, although isozyme markers are currently being used to follow the introgression of disease-resistance germplasm from *A. fistulosum* into *A. cepa* (Peffley and Mangum 1990; Cryder et al. 1991).

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In recent years polymorphic DNA markers have made a major contribution to plant improvement programmes, particularly markers based on restriction fragment length polymorphisms (RFLPs). RFLP-based linkage mapping in *Allium* species, however, presents certain problems associated with the relative ly large size of the nuclear genome relative to most other herbaceous crops. The unreplicated level of DNA per cell (2C content) in *A. cepa* is 33.5 pg compared with, for example, 2.0 pg in tomato and 7.8 pg in maize (Bennett and Smith 1976). This means that the sensitivity of the RFLP assay to reveal low-copy-number sequences in onion must be several times greater than for these other crops. Moreover, the generation of single-copy sequence probes from onion is difficult because the proportion of the genome comprising such sequences is very small. Although workers on other species were successful at targetting low-copy-number sequences by using the restriction enzyme *Pst*1 to prepare genomic libraries (Tanksley et al. 1987), attempts by us to do the same in onion were largely unsuccessuful and results in predominantly plastid sequences (unpublished).

As an alternative, random amplified DNA (RAPD) markers (Williams et al. 1990) would be useful and have advantages for genetic studies in *Allium*. In this paper we have evaluated the technique of RAPD analysis for detecting polymorphism in onion and other species of *Allium*. Our specific objectives were to determine the potential for using this approach to generate polymorphic markers in *Allium* species for genotype identification, phylogenetic analysis, and the development of a genetic map of onion.

Materials and methods

Plant material

Allium species selected from sections Cepa (*A. cepa*), Phyllodolon (*A. fistulosum*), Porrum (*A. ampeloprasum* syn. *A. porrum*) and Rhizirideum (*A. roylei* and *A. schoneoprasum*) (Vvedensky 1944) were used. *A. cepa* cultivars were chosen from as broad a genetic base as possible and included a short-day variety (Buffalo) and the following long-day varieties: Jumbo and Robot (Rijinsberger types), Cipolla di Parma (Italian), Ailsa Craig (UK), Makoi (Hungarian) and shallot (*A. cepa* syn. *A. ascalonicum*).

DNA isolation

The extraction of Allium DNA of reasonable purity and quality is difficult. Several methods were attempted [Dellaporta et al. 1983; Saghai-Maroof et al. 1984; extraction from isolated nuclei (Henfrey and Slater 1988) and a method involving gentle shearing of the cell wall and membrane to release the cytoplasm and leave a crude pellet containing nuclei from which DNA was extracted (P. Lindhout, personal communication)]. With the exception of extraction from isolated nuclei (which required fresh tissue as starting material and which resulted in a very low overall yield), all methods tried gave rise to an initial extract which was highly contaminated with polysaccharide and other impurities. A modification of the method of Saghai-Maroof et al. (1984), which included a last purification step on a caesium chloride gradient (Sambrook et al. 1989), was preferred since it gave DNA of reasonable purity and was found to be the most reliable in terms of yield.

Quantification of DNA was accomplished by analysing samples of the DNA extracts on 0.7% agarose gels alongside uncut lambda DNA standards (25–200 ng), recording an image of the gel using UVP (Cambridge, UK) gel-imaging equipment and analysing the relative intensities of the DNA bands using UVP gel-documentation software package SW2000. This method was preferred to quantification using UV absorbance at 260 nm since the crude DNA extracts contained a non-nucleic acid contaminant which absorbed strongly at 260 nm and which sometimes carried over after purification on the caesium chloride gradient.

DNA amplifications

A set of 20 random decamer oligonucleotides purchased from Operon Technologies Inc. (Alameda Calif., USA) were used as single primers for the amplification of RAPD sequences (see Table 1).

The conditions reported by Williams et al. (1990) for creating RAPD markers by PCR were optimised for use with *Allium* template DNA. Control reactions were carried out using primer A-19 in which template DNA concentration, template DNA source, magnesium concentration, and cycling conditions, were varied as detailed in the Results section. The optimum reaction mix for a 100 µl reaction comprised 50 ng template DNA, 0.2 µM primer, 200 µM each of dATP, dCTP, dGTP and dTTP, and 1 U Taq DNA polymerase (Cambio, Cambridge, UK) in 1 × Parr reaction buffer (Cambio) made up to a final concentration of 2 mM magnesium chloride. The reaction mix was overlayed with 100 µl of mineral oil. Amplification was achieved in a Techne (UK) PHC-2 thermocycler programmed as follows: a preliminary 3 min denaturation at 94 °C; 45 cycles of 1 min at 94 °C (denaturation), 1 min at 33 °C (anneal) and 2 min at 73 °C (extension); and a final extension at 73 °C for 5 min followed by a slow cooling to room temperature. Tubes containing all the reaction components except for the DNA template were included as a control for each primer used. After the cycling was completed 10 µl of the reaction products were analysed alongside small molecular weight markers VI (Boehringer Mannheim) on 1.8% agarose gels in the presence of ethidium bromide and gels were photographed under UV light.

Investigation of common bands from different cultivars

Homology between sequences representing common bands from different cultivars was confirmed by Southern analysis of RAPD gels using DNA from isolated bands as probes.

Table 1. Nucleotide sequences of random primers A-01 to A-20 (Operon Technologies Inc.)

Primer	Sequence	Primer	Sequence
A-01	5′-CAGGCCCTTC-3′	A-11	5′-CAATCGCCGT-3′
A-02	5′-TGCCGAGCTG-3′	A-12	5′-TCGGCGATAG-3′
A-03	5′-AGTCAGCCAC-3′	A-13	5′-CAGCACCCAC-3′
A-04	5′-AATCGGGCTG-3′	A-14	5′-TCTGTGCTGG-3′
A-05	5′-AGGGGTCTTG-3′	A-15	5′-TTCCGAACCC-3′
A-06	5′-GGTCCCTGAC-3′	A-16	5′-AGCCAGCGAA-3′
A-07	5′-GAAACGGGTG-3′	A-17	5′-GACCGCTTGT-3′
A-08	5′-GTGACGTAGG-3′	A-18	5′-AGGTGACCGT-3′
A-09	5′-GGGTAACGCC-3′	A-19	5′-CAAACGTCGG-3′
A-10	5′-GTGATCGCAG-3′	A-20	5′-GTTGCGATCC-3′

DNA from individual RAPD bands chosen as probes was prepared by one of the following methods.
(1) A single RAPD band was excised from the gel. A 5 µl aliquot from the excised band was added to a 100 µl reaction mix containing the appropriate primer and amplified under the conditions described above. The re-amplified DNA was purified by passage through a spin column containing Sephadex G-50 (Pharmacia), precipitated with ethanol and then redissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).
(2) The whole of the original DNA amplification reaction was fractionated on a preparative electrophoresis gel and the appropriate band excised and purified using Geneclean (Bio 101, Inc.) The DNA was radiolabelled with ^{32}P-dCTP by the random primer method (Feinberg and Vogelstein 1983, 1984).

DNA from RAPD gels was transferred to Hybond N membranes (Amersham) by capillary Southern blotting. Hybridisations were performed overnight at 65 °C in hybridisation buffer according to Church and Gilbert (1984) [1% (w/v) BSA, 1 mM EDTA, 0.5 M Na_2 HPO_4 pH 7.2, 7% (w/v) SDS]. Blots were washed at low stringency [2 × SSC, 0.1% (w/v) SDS at 60 °C] and medium-high stringency [1 × SSC, 0.1% (w/v) SDS at 65 °C] and were autoradiographed after each stringency wash using X-ray film (β-max Hyperfilm, Amersham).

Data analysis

Bands on RAPD gels were scored as present (1), absent (0), or ambiguous (*), for all the cultivars studied. Common band analysis was conducted using the computer programme Genetic Distance (Apple MacIntosh) which makes pairwise comparisons between all the cultivars evaluated to determine values of genetic distance (Rogers 1972) between them. The genetic distance was calculated as the percentage of the total number of bands scored that were clearly different between each pair of cultivars. To be scored as present the band had to be strong and/or reproducible. If there was any doubt whether a band was present or not, it was scored as ambiguous and it was treated as such by the programme. Diagrams in Figs. 1 and 2 illustrate the scoring of bands, but it should be noted that scores entered in the computer were based on replica gels, not the single photographs shown here. A total of 91 different band positions were evaluated.

This figures for genetic distance were then used as input data for cluster analysis to generate dendrograms based on (1) nearest neighbour, (2) furthest neighbour, and (3) group average, analyses (Manly 1986).

Results

Optimisation of amplification conditions for Alliums

The strategy which we used to obtain reproducible banding profiles using *Allium* DNA templates involved carrying out control reactions in which various components of the reaction mix, as well as the cycling conditions used, were varied. Assays to optimise the template concentration were conducted over the range 25–400 ng DNA per 100 µl reaction. Over the range 25–100 ng a constant band pattern was obtained but higher concentrations appeared to be inhibitory. There was a tendency for spurious (i.e., unreproducible) low-molecular-weight bands to appear at low template concentrations or when reaction mixes were vortexed, possibly due to damage to the template. At optimum template concentration (50 ng per 100 µl reaction) the band profiles remained constant using DNA template prepared by all the different extraction methods mentioned in Materials and methods, with both crude extracts and DNA purified on a caesium chloride gradient.

Two factors known to affect the stringency of PCR amplification are the magnesium concentration (which should be as low as possible) and the annealing temperature (which should be as high as possible). We found that a magnesium concentration of 2 mM was optimal since lower concentrations produced less intense amplification bands. We selected an annealing temperature of 33 °C since this was the highest temperature at which we could reliably produce amplification bands using most primers. In fact, we observed little variation in band patterns using temperatures as low as 30 °C, although an increase in non-specific amplification products was observed at lower temperatures.

Extent of polymorphism revealed in Allium by the RAPD technique

For each primer evaluated, a multiple band profile or fingerprint was produced comprising from one to five major bands plus a varying number of minor bands (see Fig. 1). With most primers the overall signal strength was good although some ambiguities arose in the scoring of minor bands. In some cases (4/20) all the bands were weak and could only be seen clearly after concentrating and loading the complete reaction products onto the gel. Bands in this category, which were weak and could only be visualized at a higher loading, were treated with caution and not included in the subsequent computer analysis. Overall the complexity of the band profiles was similar to those obtained with other plant species with smaller genomes (Williams et al. 1990; Klein-Lankhorst et al. 1991) confirming previous observations that the number of bands in RAPD profiles is independent of genome complexity (Rafalski et al. 1991).

Evaluations were performed on seven cultivars of *A. cepa,* three other cultivated *Allium* species (*A. fistulosum*, *A. schoenoprasum* and *A. ampeloprasum*) and a wild relative of onion, *A. roylei.* The latter is currently of interest as a bridging species that will allow the introduction of genes into *A. cepa* from *A. fistulosum* (de Vries, personal communication). A considerable degree of polymorphism was detected at the interspecific level with all 20 primers. Certain amplified bands appeared to be common to several species while others were present in some species but absent in others. At the intraspecific level a much lower degree of polymorphism was detected. However, polymorphic bands were seen in the profiles of *A. cepa* cultivars using 6 out of the 20 primers, for example using primer A-20 (see Fig. 1 b). Overall the frequency of scorable polymorphic bands within *A. cepa* was 7 out of a total of 57 bands analysed.

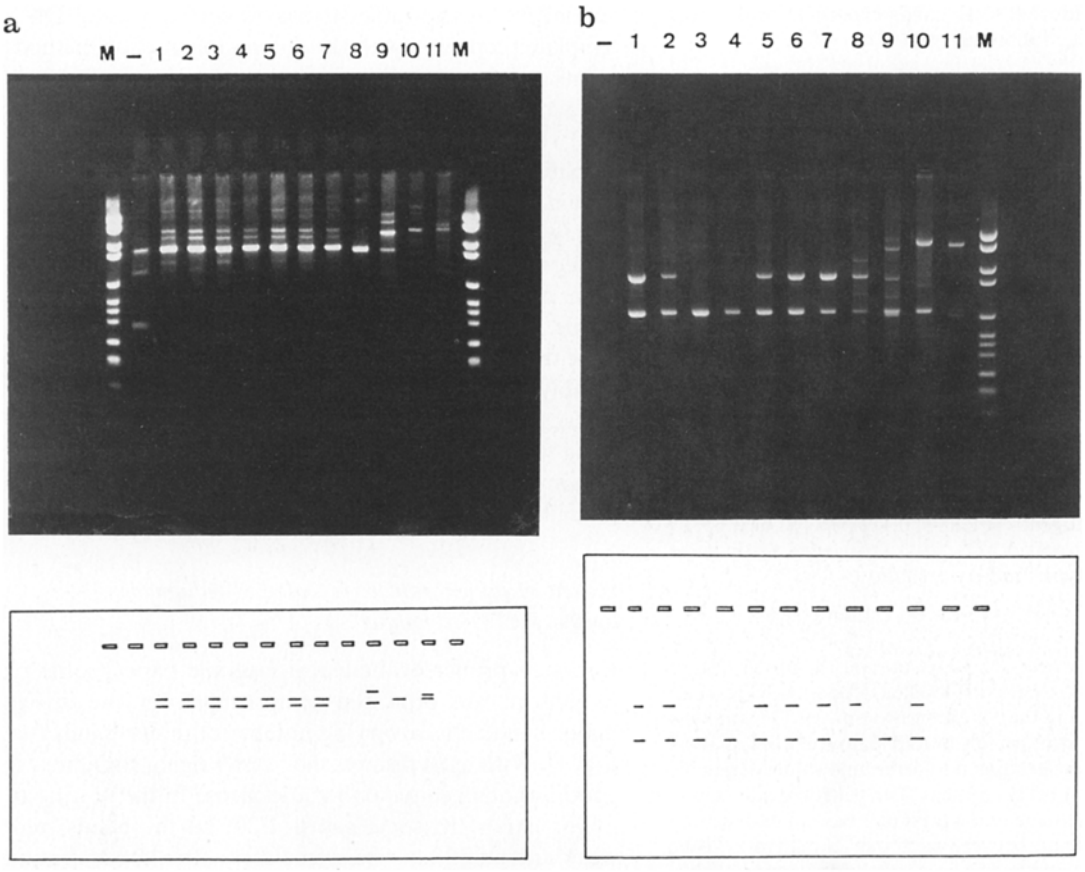

Fig. 1 a, b. Single primer PCR on total genomic *Allium* DNA using random primers A-19 (**a**) and A-20 (**b**). Photographs of gels stained with ethidium bromide (top) and diagrams showing bands scored as present and used in the computer analysis (below). *Lanes* 1–7: *A. cepa* cvs Cipolla di Parma (*lane 1*), Robot (*lane 2*), Buffalo (*lane 3*), Jumbo (*lane 4*), Makoi (*lane 5*), Ailsa Craig (*lane 6*) and shallot (*lane 7*), *lane 8*, *A. fistulosum* cv Bunching Savel; *lane 9*, *A. schoenoprasum*; *lane 10*, *A. roylei; lane 11*, *A. ampeloprasum*; *M*, Boehringer molecular weight marker VI; —, zero template control

Analysis of common bands from Allium species

The balance of common and unique bands between *Allium* species indicated that useful phylogenetic information could be gained from an analysis of common bands. However, before testing this hypothesis, it was important to establish whether bands which co-migrated in different species represented homologous sequences, and were genuinely common, or were simply fortuitous bands of similar size. Therefore, a sample of seven different bands was investigated by Southern analysis of RAPD gels using single isolated bands as probes. In order to derive information on the degree of homology between common bands, blots were washed at two stringencies, low and medium-high.

Common bands were shown to represent homologous sequences in all seven cases. An example is shown in Fig. 2. For 3 out of the 7 bands tested, the probe hybridised to all the common bands at both low and high stringency and the signal strength corresponded to that seen on the ethidium bromide-stained gel (for example, band A-11A, Fig. 2b). In the remaining cases, bands could only be confirmed as common by Southern analysis at low stringency, and a significant reduction in signal strength was observed when a band from one species was hybridised to corresponding bands from other species. This is illustrated in the case of band A-11B from *A. roylei* which gives a very strong signal when hybridised to itself but a weaker signal when hybridised to corresponding bands in *A. fistulosum*, *A. ampeloprasum*, and *A. cepa* cv Buffalo. Variations in the band intensity between common bands from different species could result from copy-number polymorphisms or, alternatively, from differences in overall amplification efficiency. Where common bands of similar intensity on the gel give hybridisation signals of different strength, it is most likely to indicate differences in sequence homologies. The results of the hybridisation experiments indicate that, whilst in some cases a high degree of homology exists between corresponding amplified sequences from different species, in other cases some sequence differences arise resulting in a weaker hybridisation signal. Indeed the

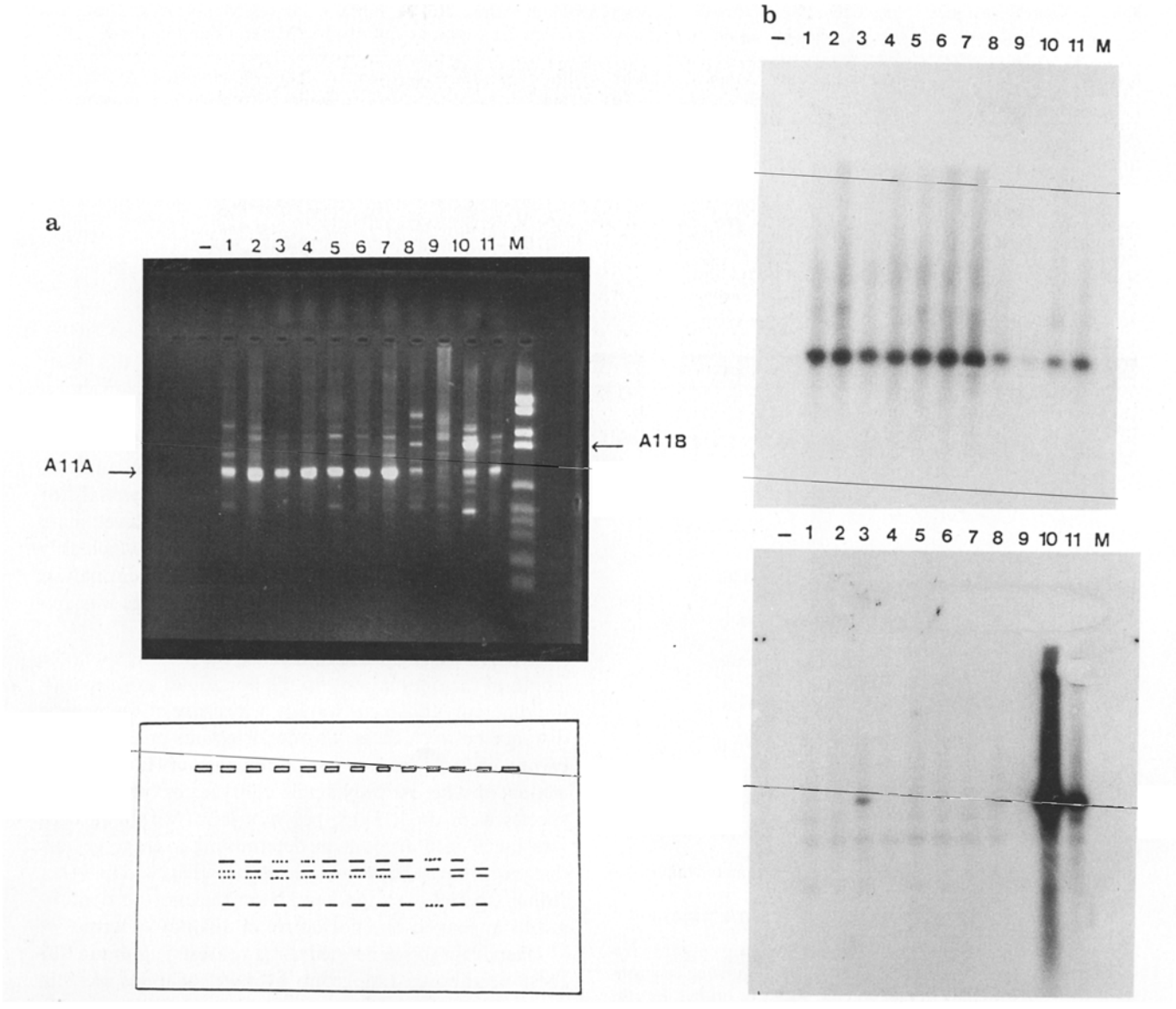

Fig. 2a, b. Confirmation of common bands between *Allium* species using primer A-11. **a** Photograph of gel stained with ethidium bromide (top) and diagram showing bands scored as present, ——, and used in the computer analysis, or ambiguous, – – –, (bottom). Lane designations as in Fig. 1. *Arrows* indicate bands A-11A (*lane 1*, *A. cepa* cv Cipolla di Parma) and A-11B (*lane 10*, *A. roylei*) excised from the gel for further amplification and radiolabelling for use as probes. **b** Autoradiographs of Southern blot prepared from gel in **a** probed with band A-11A after medium-high-stringency wash (top) and band A-11B after low-stringency wash (bottom)

relative signal strengths give an indication of the degree of homology between species for a particular DNA sequence. Thus, in the case of band A-11B there would appear to be a greater degree of homology between *A. roylei* and *A. ampeloprasum* than between *A. roylei* and *A. fistulosum*. The band from Buffalo DNA corresponding to band A-11B is hardly visible on the ethidium bromide-stained gel (Fig. 2a, track 3) and so the relatively strong signal on the corresponding autoradiograph would suggest a high degree of homology between the sequences from *A. roylei* and *A. cepa* cv Buffalo.

Common bands were scored as present, ambiguous, or absent and the data used to calculate values of genetic distance between all the cultivars studied. The results are given in Table 2. The genetic distance scale runs from 0 (identical) to 100 (different for all criteria studied) and a figure of 45 or more differentiates different species. Values of ten or less were obtained between all cultivars of *A. cepa*, the largest value arising between Jumbo and shallot (*A. cepa* syn. *A. ascalonicum*). In contrast, values obtained between cultivars of *A. cepa* and other *Allium* species range from 45 (the distance between shallot and

Table 2. Genetic distance values between cultivars of *A. cepa* (Cipolla di Parma, Robot, Buffalo, Jumbo, Makoi, Ailsa Craig and shallot), *A. fistulosum*, *A. roylei*, *A. schoenoprasum* and *A. ampeloprasum* calculated as described in Materials and methods

Variety	C di Parma	Robot	Buffalo	Jumbo	Makoi	Ailsa Craig	Shallot	A. roylei	A. fistulosum	A. ampeloprasum	A. schoenoprasum
C di Parma	0										
Robot	7	0									
Buffalo	7	5	0								
Jumbo	9	1	2	0							
Makoi	7	3	3	3	0						
Ailsa Craig	3	3	3	5	3	0					
Shallot	5	9	9	10	8	5	0				
A. roylei	48	49	48	49	51	50	45	0			
A. fist.	59	60	63	62	58	61	63	63	0		
A. ampelopr.	74	71	70	71	74	71	74	78	86	0	
A. schoenopr.	77	75	76	76	73	74	74	75	75	95	0

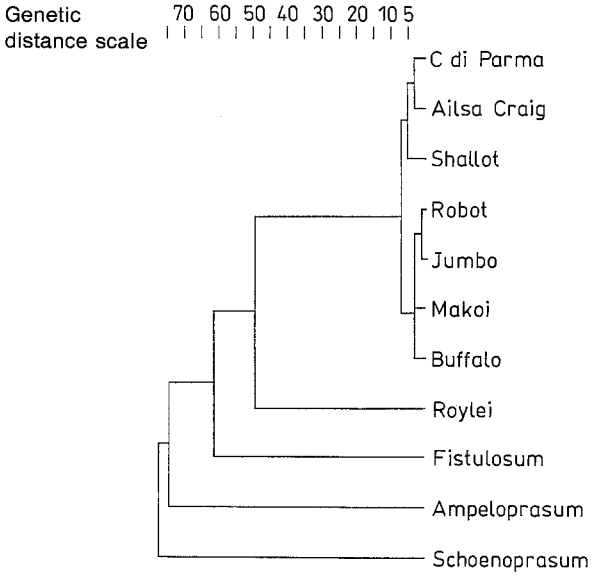

Fig. 3. Dendrogram generated by cluster (group average) analysis of genetic distance values given in Table 2 showing relationships between different *Allium* species. Relative branch lengths indicate relative genetic distances between taxa

A. roylei) upwards. The ranges of values obtained between cultivars of *A. cepa* and *A. roylei* (45–51), *A. fistulosum* (58–63), *A. ampeloprasum* (70–74) and *A. schoenoprasum* (73–77) would suggest that, of all the species studied, *A. roylei* is the closest relative to *A. cepa*.

Cluster analysis of the genetic distance values was conducted to generate dendrograms indicating relationships between the *Allium* cultivars studied (see Fig. 3). Cluster analysis is a standard method for analysing the relatedness of individuals (and hence grouping them) from measured data and has been used, for example, to study genetic diversity in maize using RFLP data (Melchinger et al. 1992). Cluster analysis has the advantage over some other grouping methods, for example principal component analysis, that the number of related groups in the material under study does not have to be known, or suspected, in order to carry out the analysis. The main assumption made is that two individuals, or cultivars, which group together at a particular level, share a common ancestor more recently than those which only join at a high level. An example of using cluster analysis to determine evolutionary relationships in dogs was given by Manly (1986).

In the dendrograms produced in the present study the length of the bifurcations on the horizontal axis separating different *Allium* cultivars is a measure of the genetic distance between them. The dendrograms are skewed in favour of *A. cepa* since seven cultivars of *A. cepa* were evaluated whereas only single cultivars of other *Allium* species were used. Thus, polymorphism within *A. cepa* was taken into account in determining average genetic distance values between species but that within other *Allium* species was ignored. Nevertheless, the dendrograms appear to be informative in qualitative terms.

Dendrograms generated using nearest neighbour, furthest neighbour, and group average, analyses were in general agreement with one another and with the previously implied classification of the genus *Allium* (Vvedensky 1944; Traub 1968; Havey 1991) apart from the case of *A. roylei*. In all three analyses *A. roylei* appears as the closest relative of *A. cepa*, followed by *A. fistulosum* and, further away, *A. ampeloprasum* (leek) and *A. schoenoprasum* (chives). The order of *A. schoenoprasum* and *A. ampeloprasum* is reversed in the nearest neighbour analysis compared to the other analyses (data not shown). However, the difference in branch length between these two species and *A. cepa* is very small and may be insignificant.

The relationships revealed between cultivars within *A. cepa* may be less reliable as they are based on an analysis of a relatively small number of polymorphisms. The two Rjinsberger types, Jumbo and Robot, emerge as the most closely related and there appears to be a grouping of the newer varieties, Buffalo, Jumbo, Makoi and Robot, away from the older varieties, Ailsa Craig and

Cipolla di Parma. Shallot, a multi-centre onion, is differentiated from conventional single-centre types. The classification of shallot within *A. cepa* is clearly supported.

Discussion

This study has demonstrated that the RAPD technique can be successfully applied to species with very large genomes like onion and other *Allium* species to reveal useful DNA polymorphisms. Thus, if results from onion are compared with those from other species, it is observed that RAPD profiles do not appear to become more complex with increasing genome size. This means that the technique is as readily applicable to species with large genomes, like onions and conifers (Carlsen et al. 1991), as to species like tomato (Martin et al. 1991; Klein-Lankhorst et al. 1991) and *Arabidopsis* (Reiter et al. 1992), where the genome is smaller.

The observation that the complexity of RAPD profiles is independent of the size of the genome is difficult to explain. Rafalski et al. (1991) have postulated that in RAPD reactions, the composition of the amplification products is determined by a competition between potential priming sites in the template rather than by the total number of priming sites available. Some mis-matching between primer and template is likely to occur under the low-stringency annealing conditions employed during the thermal cycling (Williams et al. 1990). Thus, where alternative priming sites occur, some perfectly matched and others including a degree of mismatching, competition results in a larger number of initiations from perfectly-matched sites and a reduced number of products (Ruano et al. 1991). The presence of bands of varying intensity in RAPD profiles supports this argument. However, another factor may be that the resolution and sensitivity achieved by agarose-gel electrophoresis and ethidium bromide staining merely serve to reduce the apparent complexity of the band profile. With *Allium* this is an advantage. A recently published modification of the RAPD technique used high-sensitivity silver-stain detection of amplified bands in acrylamide-urea gels (Caetano-Anolles et al. 1991). This resulted in much more complex band profiles and appeared to show that band complexity was indeed a function of genome size.

Another factor affecting the ease with which RAPD technology can be applied in species of *Allium* is the frequency of polymorphism revealed by the technique. In this study, polymorphisms within *A. cepa* were revealed by 6 of the 20 primers evaluated with a polymorphic band frequency of 7 in 57. This result suggests that, despite the intensely selected nature of modern onion cultivars, the incidence of RAPD-type polymorphism is reasonably high. RFLP studies in a wide range of species have demonstrated that considerable variation is found in the frequency of polymorphism from one species to another. Thus, for example, maize, an outbreeder, is highly polymorphic whilst the self-pollinating tomato is much less polymorphic (Helentjaris et al. 1986). Early RAPD results have pointed to the efficiency of the technique at revealing polymorphism, with success in previously recalcitrant crops like tomato (Klein-Lankhorst et al. 1991; Martin et al. 1991) and now onion, although no polymorphism has as yet been found within the cultivated peanut (Halward et al. 1992). Work is now in hand to evaluate further the use of the RAPD markers which we have found for genetic mapping in *A. cepa*.

At the interspecific level a considerable degree of polymorphism was revealed in *Allium* by the RAPD technique and the polymorphisms observed were successfully scored and used in common-band analyses similar to those applied in other crops using RFLPs (Kochert et al. 1991; Wang et al. 1992) and RAPDs (Halward et al. 1992). The analyses were based on data from 91 different band positions. Nei (1978) stated the advantages of using a large number of loci in estimations of genetic distance and recommended that at least 50 loci should be studied. The present analysis, based on nearly twice this number of loci, should, therefore, be relatively reliable at least in qualitative terms. The values of genetic distance obtained and the dendrograms produced from them appear to be informative at indicating relationships between the species studied.

A. roylei emerges as the closest relative of *A. cepa*, followed at some distance by *A. fistulosum*. This result is of significance to onion breeders currently engaged in the introgression of disease resistances and other useful traits from *A. fistulosum* into *A. cepa*. Strong interspecific crossing barriers exist between *A. cepa* and *A. fistulosum* (van der Valk et al. 1991). This is reflected in the genetic distance between them. *A. roylei*, however, would seem to be well placed to act as a bridging species, as it is more closely related to *A. cepa* than is *A. fistulosum*. Both *A. cepa* and *A. fistulosum* apparently cross with *A. roylei* (de Vries, personal communication). According to previous classifications of Vvedensky (1944) and Traub (1968), *A. roylei* falls within the large and complex section Rhizirideum which also encompasses chives (*A. schoenoprasum*) and Chinese chives (*A. tuberosum*). Our results suggest that *A. roylei* would be more appropriately classified within the section Cepa. In other respects our dendrograms are consistent with previous classifications of the species studied and closely parallel the results of Havey's (1991) study on *Allium* species based on an analysis of chloroplast RFLPs.

Our study has demonstrated that an analysis of RAPD markers can be used successfully to study phylogenetic relationship among species of *Alium*. Examination of a wider range of cultivars within each species, as well as other wild relatives of cultivated *Allium*, would be desirable and would allow the generation of a less biased

and more detailed dendrogram. It would also allow a more quantitative assessment of genetic distances between species. Such an analysis, together with data from other cladistic methods, could thus be used to make a more accurate reconstruction of the genus *Allium*. Furthermore, such an approach might be helpful in identifying other closely related wild *Allium* species of potential value in genetic improvement programmes.

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