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A genetic map of an interspecific cross in *Allium* based on amplified fragment length polymorphism (AFLP™) markers

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Abstract Segregation of 692 polymorphic AFLP™ (amplified fragment length polymorphism) fragments was determined in an F_2 of the interspecific cross *A. roylei* x *A. cepa*. Two different enzyme combinations were used, *Pst*I/*Mse*I and *Eco*RI/*Mse*I; in the latter one extra selective nucleotide was added to the *Mse*I primer. The map based on *A. cepa* markers consisted of eight linkage groups with 262 markers covering 694 cM of the expected 800 cM. The map based on *A. roylei* markers comprised 15 linkage groups with 243 markers and had a length of 626 cM. The two maps were not integrated, and 25% of the markers remained unlinked. One of the alliinase genes and a SCAR marker linked to the disease resistance gene to downy mildew are present on this map. Of the AFLP markers, 50—80% were polymorphic between *A. cepa* and *A. roylei;* the level of polymorphic markers between different *A. cepa* accessions was 4-8%.

Key words AFLP · *Allium roylei* · *Allium cepa* · Downy mildew · Genetic linkage map · Onion

Introduction

Allium roylei Stearn (2n=2x=16) is a wild relative of onion (*A. cepa* L. 2n=2x=16). It harbours several important characters such as disease resistances against downy mildew (*Peronospora destructor*) (Kofoet et al. 1990; Kik et al. 1997), leaf blight (*Botrytis squamosa*) (de Vries et al. 1992a) and anthracnose (*Colletotrichum gloeosporioides*) (Galvan et al. 1997). Furthermore, *A. roylei* restores cytoplasmatic male sterility (CMS type T) of the Rijnsburger onion, *A. cepa* (de Vries and Wietsma 1992). The exploitation of the genetic variation present in *A. roylei* for the breeding of onion has been shown to be possible because the cross between *A. cepa* and *A. roylei* yields a fertile interspecific hybrid (van der Meer and de Vries, 1990) which can be backcrossed to onion, resulting in a fertile population (Kofoet et al. 1990).

Even with the introduction of many novel marker systems during the last 10 years reports of genetic mapping in onion have been very scarce. Only recently King et al. (1998) presented a low-density genetic map based on an intraspecific onion cross and restriction fragment length polymorphisms (RFLPs). Reasons for the delay in molecular marker studies in onion are the biennial nature of onion, its severe inbreeding depression (Jones and Davis 1944) and its huge genome (Labani and Elkington 1987). Large genome sizes make molecular analyses more laborious and are the reason that high amounts of very pure DNA are required for detection of RFLPs in onion (King et al. 1998). To generate a sufficient number of molecular markers with smaller amounts of less pure DNA one can choose between polymerase chain reaction(PCR)-based methods like random amplified polymorphic DNAs (RAPDs), simple sequence repeats (SSR), amplified fragment length polymorphisms (AFLPs) and selective amplification of microsatellite polymorphic loci (SAMPL) (Witsenboer et al. 1997). While RAPDs have been used successfully for genetic studies in *Allium* (Bradeen and Havey 1995; van Raamsdonk et al. 1997; de Vries et al. 1992b; Wilkie et al. 1993), the size of the genome causes many problems, such as rather poor reproducibility and high backgrounds. SSR markers, also called microsatellite markers, are codominantly inherited and reveal high levels of polymorphisms. At present, SSR markers are being developed for onion (Fischer and Bachmann 1998), but they are not available yet. In this study we chose to use the AFLP marker (Vos et al. 1995). Genetic maps based on AFLP have been published for several species (Becker et al. 1995; Eck van

Communicated by H.C. Becker

This paper is dedicated to the memory of Wim Verbeek

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et al. 1995; Keim et al. 1997; Qi and Lindhout 1997; Wang et al. 1997). These reports show that the AFLP is a very suitable molecular marker that can be used for constructing genetic maps. AFLPs can also be used for saturating selected genomic regions with many markers (Rouppe van de Voort et al. 1997; Cnops et al. 1996).

In this report we describe the construction of a genetic map of 505 AFLP markers based on an F_2 population from an interspecific cross between *A. cepa* and *A. roylei*. The AFLP technique was also used to compare the level of polymorphisms between different onion accessions and to pinpoint two map regions, namely the region which harbours the resistance gene for downy mildew and the region in which one of the alliinase genes (a key enzyme in the sulphur metabolism) is located.

Materials and methods

Plant material

Allium roylei C502 (kindly provided by Dr. McCollum, Beltsville, USA) was used as the pollen parent in a hand cross with *A. cepa* cv 'Jumbo' (kindly provided by Novartis Seeds, Enkhuizen, the Netherlands). F_2 seed was obtained by selfing one interspecific hybrid plant using blow flies for pollination. The interspecific hybrid was maintained vegetatively and used for extra F_2 seed production in subsequent years. The F_2 population on which the AFLP marker map was based consisted of 65 plants.

DNA isolation

Genomic DNA was isolated from leaf material with a midi-DNA preparation procedure (Beek et al. 1992) with some minor modifications: after hooking the DNA out of the isopropanol mixture, the DNA was washed overnight in 70% EtOH and 100 m*M* NH_4Ac and resuspended in 200 µl or more sterile TE (10 m*M* TRIS-HCl, pH 8.0, and 1 m*M* EDTA). DNA concentrations were measured using a fluorometer. DNA could also be isolated with a miniprep DNA isolation method: approximately 0.25 g fresh leaf material was collected in Eppendorf tubes, frozen and ground in liquid nitrogen and stored at -50°C. A 750 µl aliquot of DNA isolation buffer (IB) with $Na_2S_2O_5$ (3.8 g/l) was added to the leaf material. This mixture was incubated for 60 min at 65°C with occasional inverting of the tubes [IB = lysis buffer : extraction buffer : 5% (w/v) sarkosyl (2.5:2.5:1); lysis buffer=0.2 *M* TRIS-HCl pH 7.5, 0.05 *M* EDTA, 2 *M* NaCl, 2% (w/v) CTAB; extraction buffer = 0.35 *M* sorbitol, 0.1 *M* TRIS-HCl, pH 7.5, 5 m*M* EDTA]. The DNA was further purified by adding 750 µl chloroform/isoamylalcohol (24:1), inverting the tubes (10-20 times) and centrifuging for 5 min at 14000 rpm. After transfer of the supernatant to a new tube the DNA was precipitated by the addition of 400 µl isopropanol (-20°C). The DNA could either be hooked out or it had to be pelleted for 5 min at 14000 rpm. The DNA samples were washed once with 70% ethanol and resuspended in 100 µl TE; 10 µl of the 100 µl DNA suspension was routinely used for AFLP reactions.

AFLP™ analysis

AFLP reactions for the mapping population were carried out as described by Vos et al. (1995) with two different restriction enzyme combinations: *Pst*I/*Mse*I and *Eco*RI/*Mse*I. DNA was isolated with the midi-prep method. A total of six selective nucleotides was used for the two final primers in the combination *Pst*I/*Mse*I (+3,+3) and seven selective nucleotides in the case of *Eco*RI/*Mse*I (+3,+4). For both enzyme combinations a two-step amplification procedure was conducted. In order to avoid a less stringently acting selective nucleotide (on the third position from the 3' end) the two preamplification primers with the enzyme combination *Eco*RI/*Mse*I had combined three selective nucleotides (+1,+2). The reaction mix after preamplification was diluted 1/20, and 12.5 µl was used in the final amplification. Amplified fragments were separated on denaturating polyacrylamide gels.

After the AFLP screening of the mapping population using the midi-prep method, the assesment of intra- and interspecific AFLP variation was carried out with the miniprep method and omitting the purification step in the AFLP procedure with streptavidin-coated beads.

Scoring of data and marker nomenclature

The AFLP fragments (size: 100 - 500 bp) were scored as dominant, i.e. presence versus absence of bands. Segregating markers with an unknown parental phase were discarded. All markers were scored twice, and discrepancies were researched. AFLP markers were named with the names of the two primers (e.g. P35M52) followed by a number reflecting the fragment position on the gel. The numbers given the markers are in descending molecular weight order. The name of the *Mse*I primer in the *Eco*RI/*Mse*I restriction enzyme combination is followed by the extra selective nucleotide.

SCAR analysis

Ten nanogram of DNA was used for the specific PCR reactions in a 25-µl reaction containing 0.5 U Supertaq (SphaeroQ, the Netherlands) , 100 ng primer, and 2.5 µl 10× reaction buffer (supplied by manufacturer) was added. The reaction was overlaid with one drop of mineral oil. Two primer combinations were used: a commercially available set of primers known to be linked to a gene conferring resistance to downy mildew (Kik et al. 1997) and a primer set (All-F 5' TGG GTA CTC CAA TAG CCA GTG 3'; All-R 5' TGC AAC CTT CGG AGA ACA G 3') based on the published sequence of one of the alliinase genes (Gilpin et al. 1995). Amplification was done with one step of 92°C for 5 min; 30 cycles of 92°C for 1 min, 58.5°C for 1 min and 72°C for 2 min; followed by a final extension for 10 min at 72°C. PCR products were separated on 1.5% agarose gels.

Linkage analysis

Data were analysed using JOINMAP 2.0 (Stam and van Ooijen 1995). Separate paternal (i.e. *A. roylei*) and maternal maps (i.e. *A. cepa*) were calculated. The determination of linkage groups of markers originating from *A. cepa* (cepa markers) was done with a LOD threshold of 4.0 with an occasional subdivision of a linkage group by raising the LOD threshold. The linkage groups of the markers originating from *A. roylei* (roylei markers) were determined at a LOD threshold of 3.0; again one of the groups required a subdivision using a LOD threshold of 3.5. The calculations of the linkage maps were done using all pairwise recombination estimates smaller than 0.45 and a LOD score larger than 0.001. Kosambi's mapping function was used. The correspondence of roylei linkage groups to cepa linkage groups was determined by looking for the highest LOD scores of the linkages of markers in cepa linkage groups with markers in roylei linkage groups.

Results

AFLP analysis

Thirty-three AFLP primer combinations were used to generate 692 distinctly segregating AFLP markers to-

Table 1 Polymorphic markers detected by a combination of *Eco*RI primers with three selective nucleotides and *Mse*I primers with four selective nucleotides and number of markers detected with the *Pst*I-primer in combination with *Mse*I primers, both with three selective nucleotides. Pre-amplification primers: E01= GAC TGC GTA CCA ATT CA, P01= GAC TGC GTA CAT GCA GA, M01= GAT GAG TCC TGA GTA AA M02= GAT GAG TCC TGA GTA AC. Amplification primers: E35=E01-CA, E36=E01-CC, E37=E01-CG,E38=E01-C T, P31=P01-AA, P35=P01-CA, P38=P01-CT, P43=P01-TA, M31=M01-AA, M32= M01-AC, M33=M01-AG, M34= M01-AT, M35=M01-CA, M36= M01-CC, M37=M01-CG, M47=M02-AA, M48=M02-AC, M49=M02-AG, M50=M02-AT, M51=M02-CA, M52=M02-CC, M53=M01-CG

*Eco*RI/*Mse*I	E35	E36	E37	E38
M52A	27(24)[a]	29(28)	22(20)	19(18)
M52T	46(39)			35(29)
M52C	27(26)	14/14)	20(18)	25(21)
M52G		24(19)	14(10)	14(12)
*Pst*I/*Mse*I	P31	P35	P38	P43
M31		14(3)		9(2)
M32		11(11)	9(5)	
M33	18(8)	17(14)		
M34		23(17)		
M35		17(10)		16(7)
M36		33(21)		
M37		19(11)		
M47			20(13)	25(11)
M48		11(6)	26(16)	
M49		17(10)		12(7)
M50		23(16)		
M51				27(20)
M53				12(7)

[a] The number of polymorphism markers that ended up in a linkage group of at least 4 markers are indicated in parentheses

Table 2 Putative correspondence of roylei linkage groups (R1-R15) to eight cepa linkage groups (C1–C8).

Linkage groups	
Cepa	Roylei
C1	R3, R4
C2	R2
C3	R5, R15
C4	R1
C5	R8, R9
C6	R10
C7	R6
C8	R7
??	R11, R12, R13 and R14

gether with many faint bands which were not scored. The two different enzyme combinations detected 315 markers (*Eco*RI/*Mse*I) and 377 markers (*Pst*I/*Mse*I) with 13 and 20 primer combinations, respectively (Table 1). The use of six selective basepairs (+3/+3) with the enzyme combination *Eco*RI/*Mse*I resulted in very complex patterns that were too difficult to interpret. Therefore, we chose to use one more selective nucleotide (+3/+4). The extra selective nucleotide substantially increased the interpretability of the patterns, although the number of fragments did not decrease by the expected 75%. Two extra selective base pairs (+4/+4), however, did not enable a further unravelling of the sometimes still rather complex +3/+4 patterns.

Linkage analysis

AFLP markers were scored as dominant, i.e. presence versus absence of bands. Of the clearly segregating 692 markers, 505 formed part of linkage groups consisting of at least 4 markers. Of these 505 markers, 262 originated from *A. cepa* (cepa markers) and 243 from *A. roylei* (roylei markers).

With a minimum LOD score of 4.0 the 262 cepa markers were divided into eight linkage groups. The map calculated for one of the resulting linkage groups, C4/C5, contained several negative distances and showed a bad goodness-of-fit; both are symptoms in JoinMap of attempts to integrate what are in fact separate linkage groups. Therefore, this linkage group was subdivided into two groups using a LOD threshold of 6.0. Several markers of linkage group 6A, respectively 6B, had LOD scores higher than 3.0 with several markers of the other group; combining the two linkage groups didn't show the aforementioned problems in JoinMap and therefore these two linkage groups (C6a and C6b) were combined.

The linkage groups of the roylei markers were determined at a LOD threshold of 3.0 and resulted in 13 groups. Here also, a subdivision was necessary using a LOD threshold of 3.5 for group R4/R5/R6.

Most roylei linkage groups could be tentatively assigned to cepa linkage groups (Table 2) using lower LOD scores.

The number of markers per linkage group, the length in centiMorgans, the gaps of more than 10 cM and the extent of clustering of markers are shown in Table 3. Of

Table 3 The number of markers, the length in centiMorgans, the number of gaps between markers in a linkage group of more than 10 cM, how many clusters of more than 3 markers present with no recombination between the individual markers of the clusters ($2 \times 5/1 \times 8$ means two clusters of 5 markers and one cluster of 8) and the number of *Eco*RI markers and the number of *Pst*I markers in each of the eight linkage groups (C1-C8) based on segregation of AFLP markers originating from the *A. cepa* parent and in each of the 15 linkage groups (R1-R15) based on segregation of AFLP markers originating from *A. roylei*

Cepa markers	Numbers of markers	Length (cM)	Number of gaps	Clusters	Number of *Eco*RI markers	Number of *Pst*I markers
C1	57	183	4	1×4	33	24
C2	47	78	0	1×10	31	16
C3	34	101	2	1×4/1×7	22	12
C4	32	50	0	2×5/1×8	15	17
C5	31	105	1	None	14	17
C6	27	54	0	1×4	15	12
C7	20	81	2	1×6	9	11
C8	13	42	0	None	13	0
Total	261	694	9		152	109
Roylei markers	**Numbers of markers**	**Length (cM)**	**Number of gaps**	**Clusters**	**Number of *Eco*RI markers**	**Number of *Pst*I markers**
R1	37	48	0	2×4/1×8	19	18
R2	29	40	0	1×4/1×5	17	12
R3	27	60	0	1×10	5	22
R4	24	78	2	1×6	15	9
R5	17	33	0	1×8	12	5
R6	11	44	0	None	6	5
R7	18	56	0	None	11	7
R8	17	57	1	1×6	10	7
R9	16	52	1	1×6	14	2
R10	11	28	0	None	6	5
R11	11	47	1	None	2	9
R12	9	27	0	None	4	5
R13	5	27	0	None	0	5
R14	5	14	1	None	4	1
R15	5	15	0	None	3	2
Total	243	626	6		128	114

Table 4 Number of markers with a distorted segregation pattern at a significance level of P=0.05. Skewness can be in two directions: in the direction of too many plants not having the marker (A) and in the direction of too many plants having the marker (B). The percentage of skewed segregating markers in each class is given in parentheses

	A	B
388 cepa markers	65 (17%)	35 (9%)
262 on map	45 (17%)	2 (1%)
126 unlinked	20 (16%)	22 (26%)
304 roylei markers	32 (10%)	123 (41%)
243 on map	4 (1.5%)	98 (40%)
61 unlinked	28 (46%)	25 (41%)

the *Eco*RI markers, 89% (280 of 315) ended up in linkage groups of at least 4 markers, in contrast to 59% (223 of 377) of the *Pst*I markers. More cepa markers (388) than roylei markers were scored (304); 68% of the cepa markers and 80% of the roylei markers were part of the linkage groups. Of the markers 23% were in a cluster of markers, indicating that there was no recombination between them and at least 3 other markers (Table 3). The *Eco*RI and *Pst*I markers were more or less randomly divided over the different linkage groups, although some clustering was found. The most extremes were 1 linkage group with only *Eco*RI markers (C8, 13 markers) and 1 with a majority of *Pst*I markers (R3, 22 out of 27). The cepa and roylei linkage groups are shown in Fig. 1.

Marker segregation

For dominant markers the numbers of homozygous cepa, heterozygous, and homozygous roylei markers can not be determined with direct counting, but the observed numbers of homozygous recessive genotypes can be used to make an estimate of the three classes. In total, 692 cepa and roylei markers were analysed. On average, the cepa markers were not amplified in 28% of the F_2 plants (these loci were homozygous roylei); similarly, the roylei markers were on average not amplified in 16% of the F_2 plants (homozygous cepa loci). The contribution of cepa and roylei alleles in the F_2 population is therefore 44% and 56%, respectively, and the ratio between homozygous cepa, heterozygous and homozygous roylei is 1:3.6:1.8. For all individual F_2 plants the ratio between homozygous cepa loci (cc), heterozygous loci (cr) and homozygous roylei loci (rr) was calculated. The

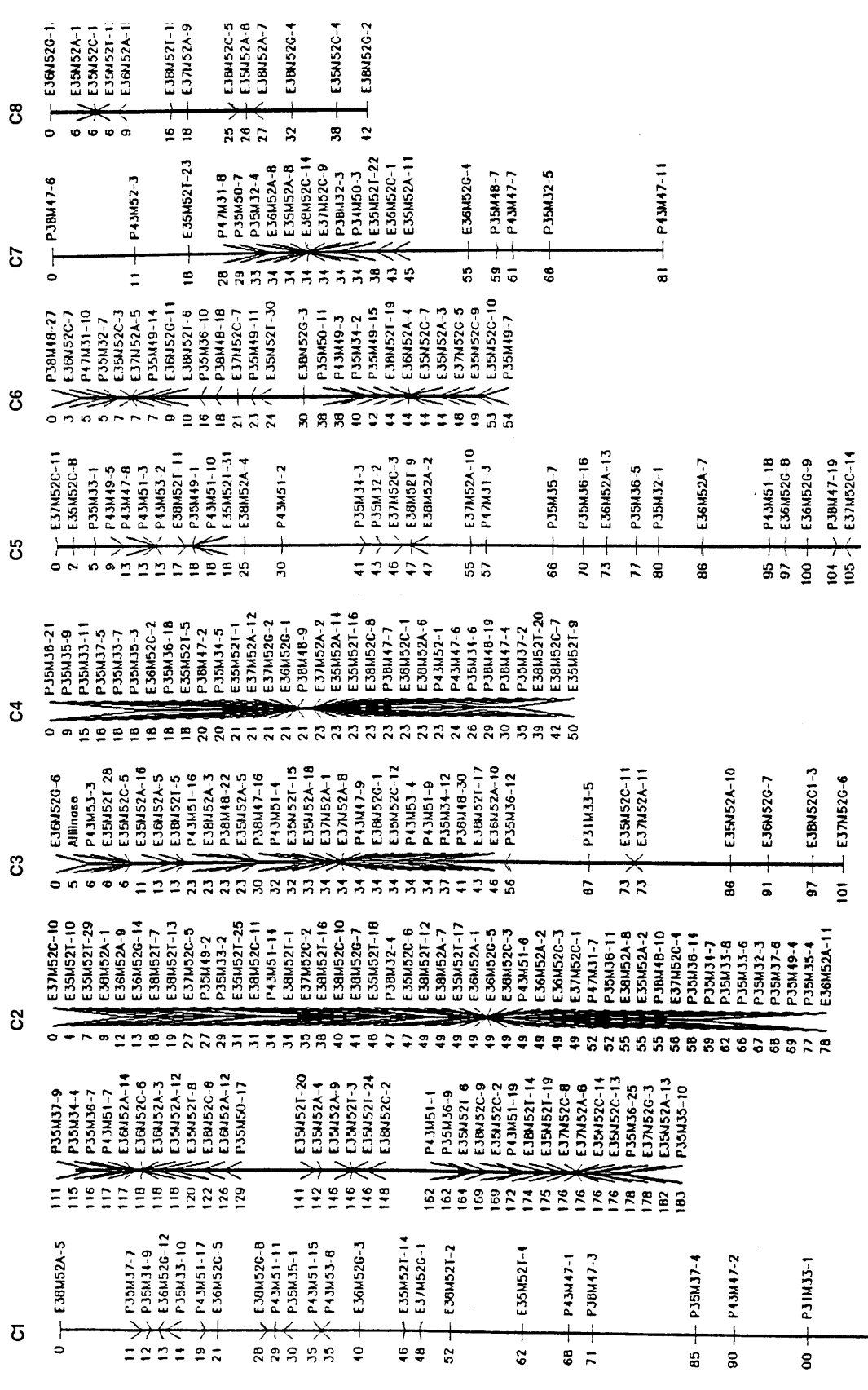

Fig. 1 a, b Linkage groups based on an F_2 population of the interspecific cross *A. cepa* x *A. roylei*. Linkage groups based on cepa-markers (**Fig. 1a,** *C1-C8*) and linkage groups based on roylei markers (**Fig. 1b**, *R1-R15*) were not integrated

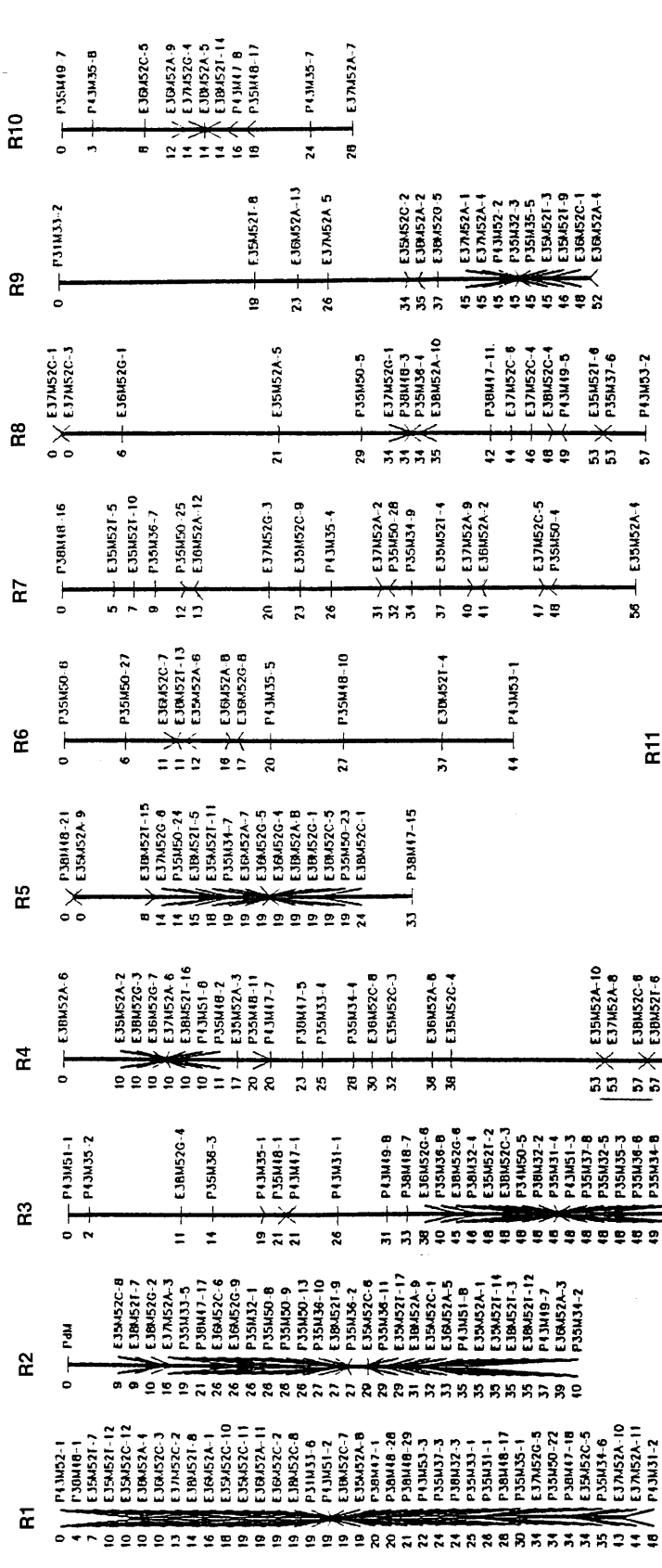

Fig. 1 b

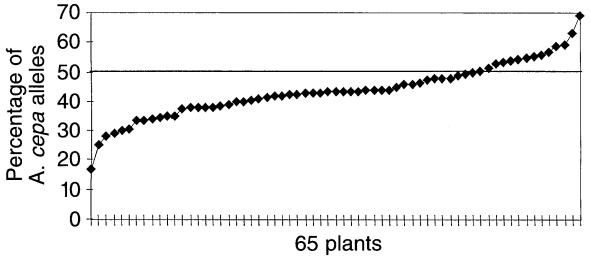

Fig. 2 The percentage of *A. cepa* alleles in individual F_2 plants sorted from low to high in a population of 65 plants

percentage of cepa alleles varied between plants from 15% to 68% (Fig. 2).

Significantly skewed segregation was observed for 100 of 388 (26%) cepa markers and for 155 of 304 (51%) roylei markers ($P < 0.05$). Of the skewed cepa markers, the majority (65 of 100) were skewed towards an excess of the roylei genotype (rr). Of the 155 skewed roylei markers 123 were distorted towards a shortage of the cepa genotype (cc). Cepa markers and roylei markers skewed in the opposite direction as the majority of the skewed markers remained almost always unlinked (class B for the cepa markers and class A for the roylei markers, Table 4). The segregation distortion was localised mainly on linkage groups C1, C2 and C5 and in R2, R4, R5, R9, R11, R12 and R13.

All 48 unlinked markers with an excess of plants not having the marker (Table 4, class A for both cepa as roylei markers) were *Pst*I/*Mse*I markers.

Specific PCR markers

Two specific PCR markers were placed on the AFLP map. A commercially available roylei-specific PCR marker (PdM) known to be linked to the downy mildew resistance gene was located on the top of linkage group R2. It was most closely linked with the marker E35M52C-8 (9 cM, LOD=4.9) With primers designed for a known sequence of alliinase, a fragment was amplified in *A. cepa* and not in *A. roylei*. This alliinase gene was mapped on cepa linkage group 3 (C3) at a 1-cM distance from AFLP markers E35M52T-28 (LOD 15.8) and E35M52A-16 (LOD 10).

Level of polymorphisms between *A. cepa* accessions compared to that of *A. cepa* and *A. roylei*

DNA that was extracted with the miniprep method was used in an AFLP procedure without the use of the streptavidin beads purification step. This resulted in better AFLP patterns with a reduction of faint bands. An average of 50 and 70 fragments were obtained with the primer combinations *Pst*I/*Mse*I and *Eco*RI/*Mse*I, respectively: 28% of these were common bands, 33% were roylei specific and 38% were cepa-specific. The percentage of polymorphic bands between the different *A. cepa* accessions, namely cvs 'Jumbo', 'Yellow Sweet Spanish', 'Sweet onion', 'Vsetatska', 'Bessanovski' and shallot cv 'Santé' was substantially lower and was 6% on average (4% for *Eco*RI markers and 8% for *Pst*I markers).

Discussion

Map construction

This is the first report on the construction of an *Allium* map based on an interspecific cross using the AFLP marker technology. The mapping effort resulted in eight cepa linkage groups and 15 roylei linkage groups with over 500 markers. The male and female maps were not integrated. The available marker data did not allow the merging of the roylei markers into the expected eight linkage groups, i.e. one for each chromosome. This was due to the distorted segregation of roylei markers in the direction of an excess of plants having the marker. Dominantly segregating roylei markers can be placed in two classes: homozygous *A. roylei* / heterozygous (rr/rc) and homozygous *A. cepa* (cc). For map construction the second class is the most informative one, and for the cepa markers this is the class with the homozygous *A. roylei* genotypes (rr). On average there were almost twice the number of individuals in class rr as in class cc. With low numbers in the most informative class the test for linkage between two groups of markers will quickly drop below the significance threshold.

Based on a chiasmata frequency of 19.4 in onion a map length of approximately 1000 cM is expected (Labani and Elkington 1987); with a chiasmata frequency of 16, as observed in our cross (Vries de et al. 1992b), a map length of around 800 cM is expected. Our cepa and roylei maps each cover about 660 cM or around 80% of the total genome. A larger population size and the use of other markers can fill up gaps and may identify markers in regions with a relatively low number of AFLP markers. Codominantly inherited markers such as microsatellites and sequence-characterized amplified regions (SCARs) will be used to integrate the cepa and roylei linkage groups. Microsatellite markers are currently developed for *Allium* and will be located on the *A. roylei* x *A. cepa* linkage map. Another possibility to construct bridges is to design primers based on known onion sequences (mostly based on sequences of cDNA probes) (King et al. 1998) and the development of codominant PCR markers (SCARs). Such markers will possibly also enable us to align the RFLP map of King et al. (1998) and our map. On our AFLP map no obvious clustering of markers was found, and *Pst*I and *Eco*RI markers were both randomly distributed over the different groups. In total, more cepa markers (388) were found than roylei markers (304). This difference might be caused by the difference in DNA content of the two species (28.5 pg in *A. roylei* vs 1C 33.5 pg per in *A. cepa*) (Albini and Jones 1990).

Distorted segregation of markers and unlinked markers

Markers in crosses between domesticated species and wild species often show skewed segregation ratios (Zamir and Tadmor, 1986). Alleles of the wild species *A. roylei* were found to have a larger chance of ending up in an F_2 individual than alleles originating from *A. cepa* (56% vs 44%). However, a large variation in the percentage of cepa alleles between individual F_2 plants was found (Fig. 2). On the basis of these results it becomes feasible to speed up a breeding programme to introgress desirable *A. roylei* genes into the onion genome by selecting those genotypes which harbour a low percentage of wild alleles but which possess the desirable trait.

AFLP markers can remain unlinked in cases where they are the result of two simultaneous, segregating amplifications in duplicated regions because two loci will be analysed as if they are one. Two unlinked AFLP markers from duplicated regions analysed as a single marker are expected to segregate 1:15 (absence/presence of fragment) or in other ratios when loosely linked duplications are involved. King et al. (1998) showed a high level of duplication in onion. Another reason for markers to remain unlinked might be the methylation sensitivity of the *Pst*I restriction enzyme. Two observations support this latter possibility: the much higher level of unlinked loci of the *Pst*I markers compared to the *Eco*RI markers (43% vs 12%) and the fact that all markers (48; Table 4) skewed in the direction of too many plants not having the marker were all *Pst*I markers.

Specific PCR markers

Two specific PCR markers were mapped. A known alliinase sequence was used to design primers. These primers were specific for only one of the alliinase genes in *A. cepa*, and this gene was mapped. The region of the resistance gene against downy mildew (*Peronospora destructor* Berk.) was pinpointed using a PCR marker (PdM) linked to this gene. No disease tests were done in this population. With Bulked Segregant Analysis (BSA, Michelmore et al. 1991) and AFLPs it will be possible to saturate this region with more markers. These markers and a disease test in a large F_2 population will enable a detailed mapping of the resistance gene against downy mildew. Subsequently, these AFLP markers can be transformed into more user-friendly PCR markers. *A. roylei* is also known to harbour other disease resistances like the ones to onion leaf blight and to anthracnose. Because the biotests for evaluating the impact of the aforementioned diseases are based on a quantitative scale, it is necessary to split every F_2 individual vegetatively or to use F_3 lines to obtain a more precise estimation of the susceptibility of each F_2 individual. The rather indistinct disease ratings and the polygenic character of the resistances make it also necessary to evaluate a larger F_2 population than the current 65 plants to pinpoint the gene(s) determining the resistances. However, individually vegetatively split F_2 individuals allow a preliminary search for markers for other resistance genes in the mapping population used in this study.

In summary, the results of this study show that the AFLP marker technology can be used to construct a genetic linkage map in *Allium* with its large genome size. Furthermore, this map will facilitate to a great extent future breeding programmes aiming at the introgression of desirable agronomic traits from the rich gene reservoir *A. roylei* into *A. cepa*.

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