Microsatellite-AFLP for genetic mapping of complex polyploids

Emidio Albertini, Andrea Porceddu, Gianpiero Marconi, Gianni Barcaccia, Luca Pallottini, and Mario Falcinelli

Abstract: In spite of the economical relevance of polyploid crops, genetic mapping of these species has been relatively overlooked. This is because of intrinsic difficulties such as the uncertainty of the chromosome behavior at meiosis I and the need for very large segregating populations. An important, yet underestimated issue, in mapping polyploids is the choice of the molecular marker system. An ideal molecular marker system for polyploid mapping should maximize the percentage of single dose markers (SDMs) detected and the possibility of recognizing allelic markers. In the present work, the marker index for genetic mapping (MI_{gm}) of M-AFLP is compared with that of AFLP and SAMPL. M-AFLPs have the highest MI_{gm} values (22 vs. 18.5 of SAMPL and 9.83 of AFLP) mostly because of their high power to detect polymorphism. Owing to their prevalent codominant inheritance, it is proposed that M-AFLP can be used for the preliminary identification of hom(e)ologous groups.

Key words: AFLP, mapping, microsatellite-AFLP, polyploids, SSR.

Résumé : Malgré l'importance économique de plusieurs espèces végétales polyploïdes, sur une base relative, moins de travaux ont été réalisés en matière de cartographie génétique chez ces espèces. Ceci découle de difficultés intrinsèques telles que l'incertitude quant au comportement chromosomique en méiose I et la nécessité d'avoir recours à de grandes populations en ségrégation. Une autre question, importante mais quelque peu sous-estimée, en matière de cartographie chez les polyploïdes concerne le choix du type de marqueur moléculaire. Un système idéal pour la cartographie chez les polyploïdes devrait maximiser le pourcentage de marqueurs à simple dose (SDM) détectés et la possibilité de reconnaître des marqueurs alléliques. Dans ce travail, les auteurs ont comparé la valeur de l'indice des marqueurs pour la cartographie génétique (MI_{gm}; « marker index for genetic mapping ») obtenue pour les M-AFLP aux valeurs du même indice pour les marqueurs AFLP et SAMPL. Les marqueurs M-AFLP présentaient les plus fortes valeurs de l'indice (22 contre 18,5 pour les marqueurs SAMPL et 9,83 pour les marqueurs AFLP). Cette valeur élevée de l'indice découlait principalement de leur capacité de détection du polymorphisme. En raison de leur hérédité principalement de type codominant, il est suggéré que les marqueurs M-AFLP pourrait être employés pour l'identification préliminaire de groupes homéologues.

Mots clés : AFLP, cartographie, microsatellite-AFLP, polyploïdes, SSR.

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Introduction

Polyploidization is an important evolutionary factor in flowering plants (Stebbins 1971; Soltis and Soltis 2000; Wu et al. 2001). Polyploids can be classified on the basis of their origin: allopolyploids are derived from the combination of distinct genomes followed by doubling, whereas autopolyploids originate from the chromosomal doubling of a species by fusion of 2*n* gametes (Stebbins 1950; Soltis and Soltis 2000). It has been proposed that 30–80% of all flowering species have experienced episodes of polyploidization (Stebbins 1971; Grant 1981; Masterson 1994). This frequency would be even higher if the count were restricted to domesticated plants (Hilu 1993). Potato, soybean, wheat, and alfalfa are examples of polyploid species of economical relevance.

Allopolyploids are considered to be much more prevalent than autopolyploids in nature, but recent genetic analyses have shown that autopolyploids are much more common than previously thought (Soltis and Soltis 2000). Although polyploids are widespread among important crops, their genetic mapping has long lagged behind that of diploids owing to several factors (Wu et al. 1992). First, the chromosome behavior of many polyploids is unclear, which makes it difficult to define the pattern of inheritance. In fact, many species with high ploidy levels may be mixtures of allopolyploids

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and autopolyploids (Wu et al. 1992). Second, in the case of autopolyploids, the ability to detect repulsion- phase linkages is low and, unless very large populations are available, mapping must be restricted to coupling-phase markers (Wu et al. 1992; Hackett et al. 1998; Ripol et al. 1999). Third, because of the large number of possible genotypes, the banding pattern displayed by most molecular markers is too complex (Grivet et al. 1996). To give an example, an autotetraploid parent, when selfed, can yield a maximum of 19 different genotypes per locus if the parent had maximum heterozygosity (four different alleles) and bivalent chromosome pairing.

Because of these intrinsic difficulties, mapping of complex polyploids is usually conducted as a multistep process (Wu et al. 1992; Hackett et al. 1998; Ripol et al. 1999; Qu and Hancock 2001). Single-dose markers (SDMs), that is, markers segregating 1:1 in an F_1 or BC_1 population, are scored and linkage analysis is limited to coupling-phase markers. This is achieved by truncating recombination fractions greater than 0.5, because of markers in repulsion, to 0.5 and and setting the corresponding LOD score to zero (Hackett et al. 1998). Hom(e)ologous groups are identified by searching for codominant markers and finally the chromosome behavior within the group is assessed by either analyzing the segregation of these codominant markers or by studying the distribution of coupling vs. repulsion markers in each pair of the homologous group (Wu et al. 1992; Qu and Hancock 2001). The information gained may then be used to carry out a new data analysis that takes into account the chromosome behavior within each homologous group.

Another important, yet underestimated, issue is the choice of the molecular marker system. An ideal molecular marker system for polyploid mapping should maximize at least two parameters: (*i*) the percentage of SDMs detected per assay and (*ii*) the possibility of recognizing allelic markers. An additional parameter is the genome coverage, i.e., the distribution of the marker in the physical map.

RFLP markers have been widely used for mapping polyploids (Da Silva et al. 1993; Da Silva et al. 1995; Xu et al. 1995; Grivet et al. 1996; Ming et al. 1998; Brouwer and Osborn 1999). Their theoretically high capacity for recognizing allelic markers is complicated in polyploids owing to the low resolution power and the presence of paralogous loci (Grivet et al. 1996). AFLP-based marker systems provide molecular assays that combine the reliability of RFLPs with the power of PCR. SAMPL (selective amplified microsatellite polymorphic locus) analysis extends AFLP technology to include amplification of microsatellite loci without prior cloning and characterization of specific microsatellite sequence (Morgante and Vogel 1994). SAMPL analysis uses one AFLP primer in combination with a primer that is complementary to the sequence of two adjacent SSRs. These molecular markers are, in general, expected to provide many SDMs per assay, but the complexity of the visualized banding pattern makes it difficult to identify allelic bands. In a previous work, we compared the efficiency of AFLP (Vos et al. 1995) and SAMPL (Morgante and Vogel 1994) in detecting SDMs in an F_1 population of *Poa pratensis* L., and showed that SAMPL was the most useful for detecting SDMs (Porceddu et al. 2002*a*). In this study, a new AFLP-derived marker system, in which markers are anchored to the 5′-end of microsatellite (SSR) loci, has been introduced into the comparison. In principle, this microsatellite-AFLP (M-AFLP, Van Eijk et al. 2001) system should combine the high multiplex ratio of AFLP-derived markers with the high heterozygosity of SSRs. Because most of the polymorphisms detected by the M-AFLP arise from variations in the number of repeat units, it seemed feasible to develop SSR-type codominat markers from polymorphic M-AFLP bands.

Table 1. List of 5′-AM primers.

Primer name	Primer sequence
I16	$CGGC(AC)_7$
I17	$GAGC(TC)_7$
I18	$GTGC(TC)_7$
I53	$CGCAA(CA)_9$
I57	$CGTCC(CA)_9$
I60	$GCCAC(GCT)_6$

Materials and methods

Plant material, controlled matings, and DNA isolation

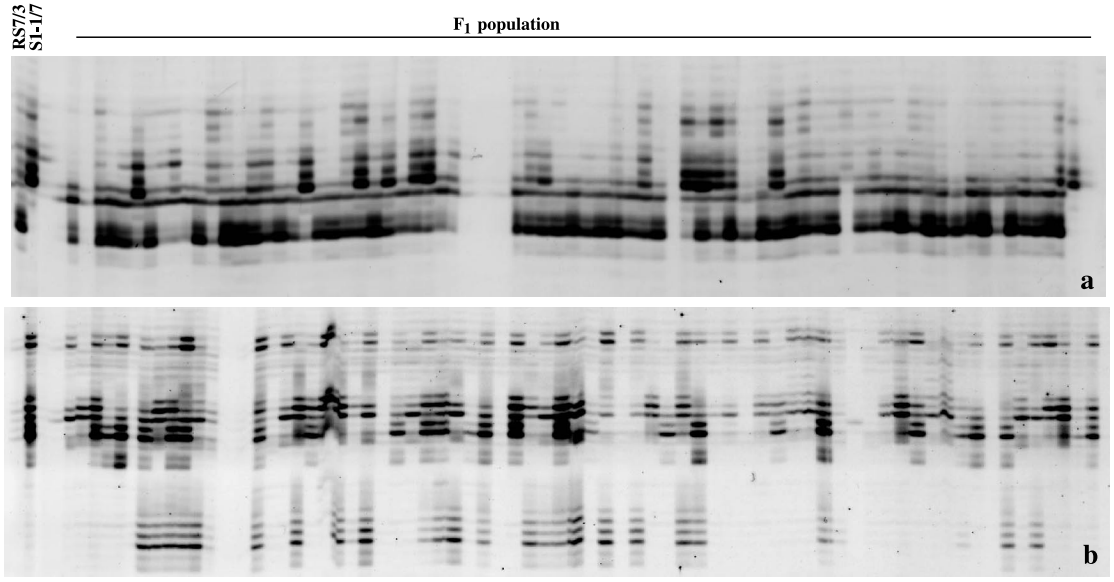
A segregating F_1 population of 68 plants was produced (Porceddu et al. 2002*a*) by crossing a completely sexual clone (Sl/1-7) with a highly apomictic genotype (RS7-3). The sexual clone was derived from a cross between two completely sexual genotypes selected from German cultivars (Matzk 1991; Barcaccia et al. 1998), while the apomictic clone was selected from a natural Italian population (Mazzucato 1995). Cytological investigations showed that the chromosome number of S1/1-7 and RS7-3 were $2n = 36$ and 64, respectively, whereas all progeny plants had a 50-chromosome complement (Porceddu et al. 2002*a*).

M-AFLP protocol and SSR-enriched library construction

Total DNA (500 ng) was restricted-ligated and pre-amplified according to Vos et al. (1995). M-AFLP amplifications were performed in a 20-μL reaction mix containing 1/100 of the pre-amplified DNA, 50 ng fluorescent-labeled *Eco*RI+3 oligonucleotide primer, 10 pmol unlabeled 5′-anchored microsatellite primer (hereafter named 5′-AM primer; Table 1), 2 μL PCR buffer (Amersham Biosciences), 0.2 mM dNTPs, 0.4 U *Taq* polymerase (Amersham Biosciences). The 5′-AM primers were designed to faithfully anchor to the 5′-end of microsatellite repeats (Table 1). After PCR, 8 μL of loading buffer (98% formamide, 2% dextran blue, 0.25 mM EDTA) were added to each tube. Samples were denatured at 90°C for 5 min and then immediately placed on ice. For each sample, 4 μL were loaded onto a 6% vertical polyacrylamide gel (60 cm × 30 cm × 0.4 mm), run for 3 h at 80 W, and then scanned using the Genomyx system (Beckman Coulter Corporation, Calif.).

An SSR-enriched library was developed starting with 6 μg of total genomic DNA as described in Jones et al. (2001).

Fig. 1. Portions of M-AFLP gels generated by (*a*) Eco+CCA–I53 and (*b*) Eco+CAC–I60 primer combinations.



Segregation and marker index for genetic mapping analysis

Only SDMs that were polymorphic between parents, that is, markers present in single copy at a given locus, were used for linkage mapping analysis. The expected segregation patterns (presence vs. absence) of marker alleles observed in the F_1 population were assayed by χ^2 analysis. In absence of segregation distortion, the expected segregation ratio for simplex markers is 1:1, irrespective of the type of chromosome pairing. The expected segregation ratio (presence vs. absence) of a double-dose marker in gametes of autopolyploids with bivalent pairing at meiosis is $(3h - 2):(h - 2)$, where h represents the ploidy level. At high ploidy levels the segregation ratio approaches 3:1 (presence vs. absence) (Sorrels 1992). Therefore, only markers with a segregation ratio lower than 1.73:1 $((1 \times 3:1)^{1/2}$; Bailey 1961) were retained. This ratio gives an equivalent χ^2 for both the 1:1 and 3:1 hypotheses the latter being the smallest theoretical ratio for all non-SDM markers (Grivet et al. 1996).

The marker index for genetic mapping (MI_{gm}) was calculated by taking the average percentage of SDMs detected per assay and multiplying it by the average multiplex ratio (average number of bands obtained per assay).

Development of SSR markers from M-AFLP bands

Selected M-AFLP fragments were excised from polyacrylamide gels, reamplified, and cloned into pCR4-TOPO vector using the TOPO TA Cloning kit for sequencing (Invitrogen, Carlsbad, Calif.). The same was done for selected clones from the library. The inserts were sequenced by Big Dye Terminator 2.0 chemistry (Applied Biosystems). Primers, specific to the sequence flanking the repeat, were designed with OLIGO software (National Biosciences) and labeled with 6-FAM, VIC, or NED (Applied Biosystems, Foster City, Calif.).

PCR amplification of the microsatellite loci

Amplification of the M-AFLP-derived-SSR loci from genomic DNA was performed using a 25-μL reaction mixture containing 50 ng of genomic DNA, 0.2 mM dNTP, 1× PCR buffer (Amersham Pharmacia, Piscataway, N.J.), 1 U *Taq* polymerase (Amersham Pharmacia), 10 pmol of 5′-AM primer, and 10 pmol of labeled locus-specific primer. The 5′-AM primer corresponded to that used to amplify the fragment from which the sequence-specific primer was derived. PCR was performed using the following profile: after an initial denaturation at 94°C for 5 min, 35 cycles at 94°C for 30 s, T_m (optimized melting-point temperature for each primer pair) for 30 s, and 72°C for 2 min, followed by a final step of 20 min at 72°C. For standard SSRs, amplification reactions were performed in a total volume of 25 μL containing the following: 2.5 μL of PCR buffer (Amersham Pharmacia), 1 U *Taq* polymerase (Amersham Pharmacia), 0.2 mM dNTP, and 10 pmol of each locus-specific primer using the same thermal profile as described above. After PCR, samples were run on an ABI Prism 377 automatic sequencer (Applied Biosystems).

Linkage mapping

M-AFLP, M-AFLP-derived SSR, and SSR markers segregating as SDM in the F_1 population were used, together with SAMPL and AFLP markers already reported by Porceddu et al. (2002*a*), to construct a linkage map using Joinmap version 3.0 (Van Ooijen and Voorrips 2001) and employing the "backcross" (BC_1) option. To identify linkage groups with selected markers, the "grouping" module was used, setting a minimum LOD score of 3.0 and a maximum recombination frequency of 0.30. Map distances, expressed in centimorgans (cM), were calculated using the Kosambi function (Kosambi 1944).

Results

In the present study, we compared the MI_{gm} of microsatellite-AFLP (Fig. 1) with that of AFLP and SAMPL. Because linkage analysis in autopolyploids is virtually restricted to single-dose markers (SDMs), the MI_{gm} can be calculated by taking the average percentage of SDMs de-

Table 2. Descriptive statistics of AFLP, SAMPL, and M-AFLP markers as estimated using data from the F_1 segregating population.

			Bands segregating in the F_1 population					
Marker system	No. of assays	E	Total (%)	Shared between parents (%)	Segregating from either parents (%)	MDMs (%)	SDMs (%)	MI_{gm}
AFLP	9	85.5	35.7	19.5	16.2	15	1.2	9.83
SAMPL	12	60.7	41.0	8	33	2.4	30.6	18.5
M-AFLP	6	34.5	71.4	4.3	67.1	3.4	63.7	22.0

Note: E, average multiplex ratio; DDM, average number of multiple-dose markers detected per assay; SDM, average number of single-dose markers detected per assay; MI_{gm}, marker index for genetic mapping.

Table 3. List of M-AFLP-derived SSR-specific primers in combination with the relative 5′-AM primer, expected product size, and number of alleles detected in the F_1 population.

Primer name	Primer sequence	5′-AM primer	Expected size	No. of alleles	m/p/s[a]
AE12	AATTCCCAATCCAATTATATTAGT	I18	280	—	—
AE14	AATTCCCAAGCTGATTCTTTCGGC	I60	160	—	—
AE17	CCCAGGTAAAAGGTCTCG	I17	190	—	—
AE18	AATTCCAAAAAGGTGCGCCACTGA	I60	135	2	2p
AE19	AATTCCACAGAAATTATGACGAGA	I17	160	5	4m/1p
AE24	AAGACCTAGCTAGCTTTA	I18	190	7	2m/2p/3s
AE26	TCGGGAGGAGATTTATTC	I57	200	3	1m/2p
AE33	TTCACTCTAAAAATCATG	I16	120	4	1m/2p/3s

[a]m, maternal; p, paternal; s, shared between parents.

Table 4. List of SSR loci with primer pair sequences, expected product size, number of alleles detected in the F_1 population and the polymorphic (P) or monomorphic (M) nature in the F_1 population.

SSR name	Primer pair	Expected size	Nature	No. of alleles	m/p/s[a]
AE1	TTTGGTGCACATAGTTGA TGGAGTCCTCTGATTTTG	380	P	9	3m/5p/1s
AE2	TGGACAAATAAGCAACTG AGAAGCACACACATACTG	170	P	3	1m/2s
AE4	AGCAGCATAACTATTTGTAGTACGT GACGCTCGTCTCCTATAAGTACCAT	210	P	4	1m/3s
AE5	TCTCCCTCATAACCTAACAGAATTA GAGAAGGCATCTGTAAATGATACAG	293	P	5	2m/1p/2s
AE6	CTGAGTCCTTCATGTATGCG TCGAGGGAGAGTGATGTA	281	M	2	—
AE9	ACTGGAAAGGAAACTACACGTC CACCGCGGACCCCTCCGGTA	150	P	6	3m/2p/1s
AE10	CGCTTTCCGGAACATTGGTC GGCGTGGGGCTGCCCGAG	237	M	3	—
AE11	TCTAGGAAGAAGGTGATATAA GCCTCGGGCAGCCCCACG	224	M	4	—

[a]m, maternal; p, paternal; s, shared between parents.

tected per assay times the average multiplex ratio. The allelic dose of bands segregating in an F_1 pseudotestcross population of *P. pratensis* was inferred by performing a χ^2 test. M-AFLP showed the highest MI_{gm} (22), followed by SAMPL (18.5) and AFLP (9.83) (Table 2). The high performances of M-AFLP and SAMPL were due to their strong preference for single-dose markers (63.7% of M-AFLPs, 30.6% of SAMPLs, and 1.2% of AFLPs were inherited as SDMs), whereas AFLP showed the highest multiplex ratio (an average of 85.5 bands for AFLP vs. 60.7 bands for SAMPL and 34.5 amplification products for M-AFLP). It is interesting to note that AFLP detected a high percentage of segregating bands but most of these were either shared between parents (19.5%) or present in multiple dose (15%).

An important aspect of genetic mapping in autopolyploids is that repulsion-phase linkages are only detectable in large segregating populations (Wu et al. 2001). The identification of allelic markers is therefore an important component of the overall usefulness of marker systems because it allows hom(e)ologous groups to be identified.

The conversion of AFLP into codominant SCAR markers has been very troublesome (Shan et al. 1999; Dussle et al.

Fig. 2. Distribution frequencies of segregation ratios (presence:absence) of the 21 M-AFLP-derived SSR and 36 SSR alleles polymorphic between parents. Segregation ratio 1.73:1 was considered the cut-off point between SDMs and non-SDMs.

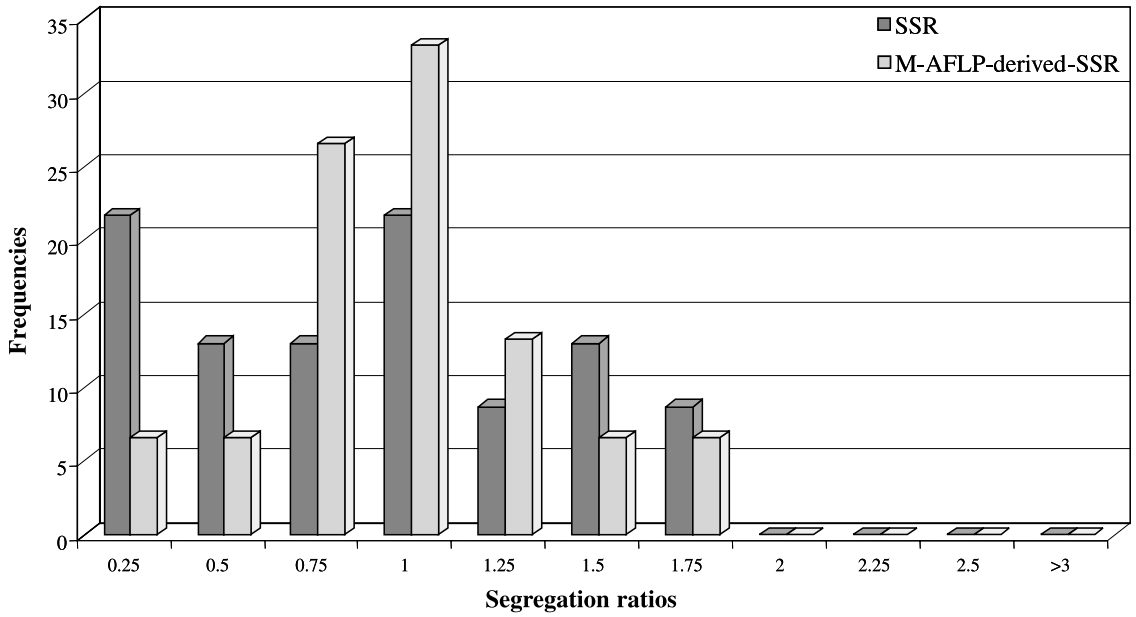
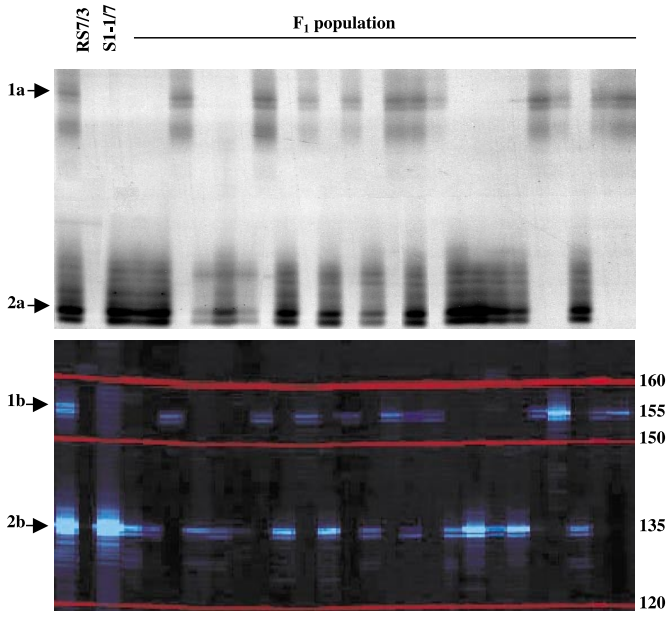


Fig. 3. The pattern generated by AE18-I60 primer combination (*b*) is identical to that relative to the segregating M-AFLP originated from Eco+CAA–I60 primer combination (*a*) from which the M-AFLP band was cloned. The cloned M-AFLP band (arrow 2*a*) cosegregated with one of the M-AFLP-derived-SSR alleles (arrow 2*b*). Note that bands indicated by arrows 1*a* and 2*a* co-segregated with the alleles of the M-AFLP-derived SSR (arrows 1*b* and 2*b*).

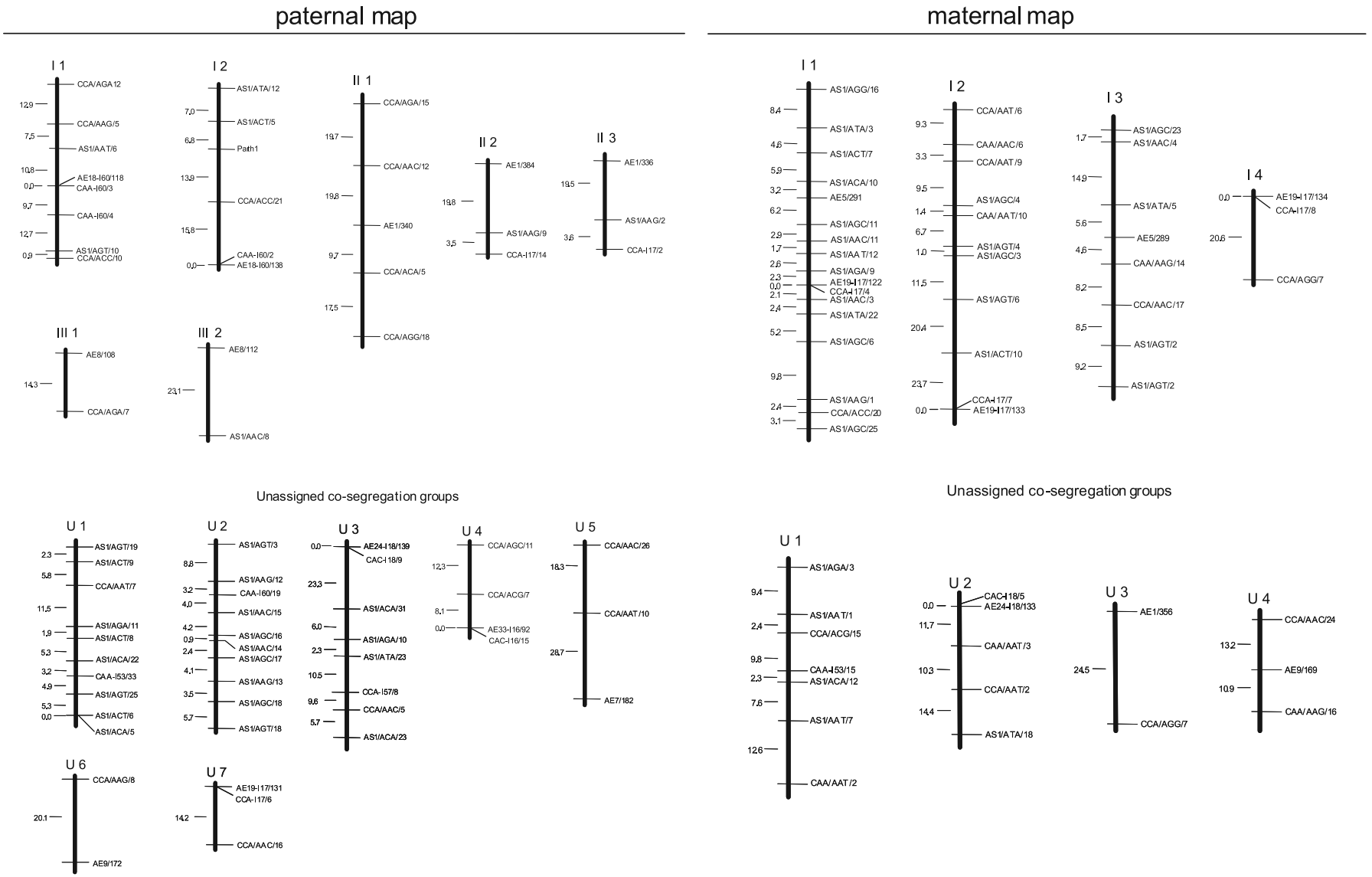


2002) and only a few successes have been reported to date (Bradeen and Simon 1998; Shan et al. 1999; Negi et al. 2000; Reamon-Büttner and Jung 2000; Meksem et al. 2001; Xu et al. 2001; Albertini et al. 2001; Dussle et al. 2002). In contrast, since the polymorphism detected by M-AFLP is presumed to arise from a variation in the number of repeat units (Vogel 1995), the transformation of M-AFLP into codominant SSR-type markers is expected to be rather efficient. To test this hypothesis, 12 bands that were polymorphic between the parents and segregated 1:1 in the F_1 population were eluted from the polyacrylamide gels, re-amplified, cloned into a TA vector, and sequenced. The expected di- or trinucleotides were retrieved from one end of each fragment. Only eight sequences had additional repetitive units; the other four bands did not contain additional units of SSR sequence, so they were not used in the analysis. Based on the internal sequence, a locus-specific primer was designed for each of the eight selected clones (Table 3). These primers were used in combination with the relative 5′-AM primer (Table 3).

M-AFLP-derived SSR primer pairs were used for locus-specific PCR amplifications. Of the eight M-AFLP-derived-SSRs, only five gave easily interpretable patterns. The remaining primer combinations produced complex profiles that were incompatible with the ploidy level. The five polymorphic M-AFLP-derived SSR primers were used on the F_1 population and produced an average of 4.2 alleles/locus. In particular, a minimum of two alleles were produced by AE18-I60 and a maximum of seven were produced by AE24-I18 (Table 3).

Since M-AFLP-derived SSRs are one-sided tailed amplification fragments of SSR loci, their average information content could be somewhat biased with respect to classical SSRs. To test this hypothesis, the information obtained from M-AFLP-derived SSRs and from classical SSRs were compared. Eleven SSR-containing genomic fragments were cloned from a $(CT)_n$-enriched genomic DNA library. For three of them, the sequence information at one end was insufficient for primer design. Locus-specific primer pairs flanking the repeats were designed for the remaining eight. Table 4 lists the sequence of primer pairs for the eight loci with the expected size of their amplification products, and the number of alleles detected in the F_1 population. The eight SSR primer pairs amplified the alleles that segregated

Fig. 4. Cosegregating groups of the paternal and the maternal genotype where either M-AFLP-derived SSR or SSR were mapped using the BC_1 option. The map also reports the groups with more than seven markers where M-AFLP markers were mapped. Distances are expressed in centimorgans and were calculated using the Kosambi function (Kosambi 1944). AFLP locus designations refer to the *Eco*RI–*Mse*I selective combination of bases, those of SAMPL refer to the *Mse*I selective combinations with the AS1 primer, whereas those of M-AFLP refer to the *Eco*RI selective bases/5′-AM primer. The M-AFLP-derived SSR loci refer to the combination of locus-specific primer and the 5′-AM primer with the relative molecular weight and those of SSR refer to the primer pair used with the relative molecular weight. M-AFLP, M-AFLP-derived-SSR and SSR loci are in bold type.



from either parent. The number of alleles amplified varied from two for locus AE6 to nine for locus AE1, with an average of 4.5 alleles per locus (Table 4).

The results of the χ^2 analyses of the segregation ratios varying from 1:4 to 5:1, presence vs. absence, in the F_1 segregating population are shown in Fig. 2. While the segregation ratios of M-AFLP-derived SSRs were mostly in classes 0.75 (1:1.3) and 1 (1:1), those of the SSRs were distributed between classes 0.25 (1:4) and 1.75 (1.75:1). This result suggests that the information derived from the M-AFLP-derived SSRs is similar to that derived from classical SSR loci.

To identify M-AFLP allelic bands, we performed a linkage analysis by combining the data from M-AFLP-derived SSRs with those of M-AFLP and SSR, alongside an existing framework of AFLP and SAMPL markers (Porceddu et al. 2002*a*).

All of the M-AFLP-derived SSRs showed a segregation coincident to the original M-AFLP band (Fig. 3). As expected, in the five mapped M-AFLP-derived SSRs, the cloned M-AFLP band was mapped at 0 cM with one of the alleles of the M-AFLP-derived SSR (Fig. 4). In three cases, the other M-AFLP-derived SSR alleles were mapped at 0 cM (Fig. 4) with other bands belonging to the same M-AFLP assay (two alleles for locus AE18-I60, four alleles for locus AE19-I17, and two alleles for locus AE24-I18 alleles overall). This finding demonstrated that most of the M-AFLP polymorphisms are derived from a variation in the number of repeat units in the SSR. Linkage groups with M-AFLPs, M-AFLP-derived SSRs, and classical SSRs are shown in Fig. 4.

Discussion

Very few reports can be found in the literature about linkage mapping of polyploids. Genetic linkage maps in cultivated sugarcane were reported by Grivet et al. (1996) and Hoarau et al. (2001). Ukoskit and Thompson (1997) reported a map of sweetpotato, Groh et al. (2001) a map of oats, and Brouwer and Osborn (1999) a map of tetraploid alfalfa. Other genetic maps, constructed for some polyploid species, e.g., alfalfa (Kiss et al. 1993; Tavoletti et al. 1996; Kaló et al. 2000; Porceddu et al. 2002*b*) and potato (Jacobs et al. 1995), have been based on closely related diploid species. It would be impossible to apply such a strategy to species that have no close diploid relatives or whose genomic constitution is poorly defined. The construction of genetic maps of polyploid species has lagged behind that of diploid species because (*i*) the statistics are far more complicated for polyploids than for diploids, (*ii*) large segregating populations are needed to obtain reliable estimates of genetic distances and (*iii*) the knowledge about the genomic constitution of most polyploids is very limited. The chromosome number of *P. pratensis* varies from a minimum of 28 to a maximum of 147. This variation in chromosome number is due to the high frequency of mitotic and meiotic aberrations and the marked degree of hybridization with other species (Clausen 1961).

Although models for mapping polyploids have recently been redefined, little information is available about the usefulness of different marker systems for genetic mapping of complex polyploids.

This study compared three AFLP-based marker systems for genetic mapping in complex polyploids.

The M-AFLP and SAMPL techniques were more informative, because they produced the highest number of SDM markers per assay. The greater efficiency was due to a greater capacity to detect polymorphisms rather than the number of loci per assay. Unlike AFLP, M-AFLP and SAMPL are anchored to microsatellite regions. Therefore, it is conceivable that the higher power of SDM detection is mostly due to variations in the SSR region. Accordingly, M-AFLP should show the highest detection power, because 5′-AM primers are anchored at the 5′-ends of microsatellites, allowing the variation to be inspected over their entire length. In contrast, SAMPL primers are anchored at the motif change of composed microsatellites and would be expected to inspect the variation in only a part of the whole microsatellite.

We have shown that most M-AFLP-derived SSRs cosegregated with the original M-AFLP bands, which indicates that the polymorphisms of M-AFLP arise, mainly, in the SSR motif.

In light of this observation, we propose that a preliminary indication of the hom(e)ology between linkage groups can be deduced by analyzing the origin (i.e., primer combination) and map position of bands on each pair of the identified linkage groups. If the marker distribution in two linkage groups is found to be similar, and corresponding markers of either group originate from the same primer combination, the two groups can be investigated as "hom(e)ologous".

Genome coverage is another important parameter for genome mapping. Since M-AFLP and SAMPL protocols are based on the AFLP procedure, their distribution should be confined to that of AFLP. However, recent analyses have shown that the frequency of microsatellites is significantly higher in transcribed regions than in non-transcribed DNA (Morgante et al. 2002). This suggests that M-AFLP should be more efficient than AFLP in tagging genes. In addition, since microsatellite frequency seems to be higher in non-repetitive DNA than in the repetitive genome fraction (Morgante et al. 2002), SSR-anchored techniques should be less prone to detect multi-copy markers that can complicate the genetic mapping of complex polyploid species.

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