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Mapping of simple sequence repeat (SSR) DNA markers in diploid and tetraploid alfalfa

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Abstract Cultivated alfalfa (*Medicago sativa*) is an autotetraploid. However, all three existing alfalfa genetic maps resulted from crosses of diploid alfalfa. The current study was undertaken to evaluate the use of Simple Sequence Repeat (SSR) DNA markers for mapping in diploid and tetraploid alfalfa. Ten SSR markers were incorporated into an existing F_2 diploid alfalfa RFLP map and also mapped in an F_2 tetraploid population. The tetraploid population had two to four alleles in each of the loci examined. The segregation of these alleles in the tetraploid mapping population generally was clear and easy to interpret. Because of the complexity of tetrasomic linkage analysis and a lack of computer software to accommodate it, linkage relationships at the tetraploid level were determined using a single-dose allele (SDA) analysis, where the presence or absence of each allele was scored independently of the other alleles at the same locus. The SDA diploid map was also constructed to compare mapping using SDA to the standard co-dominant method. Linkage groups were generally conserved among the tetraploid and the two diploid linkage maps, except for segments where severe segregation distortion was present. Segregation distortion, which was present in both tetraploid and diploid populations, probably resulted from inbreeding depression. The ease of analysis together with the abundance of SSR loci in the alfalfa genome indicated that SSR markers should be a useful tool for mapping tetraploid alfalfa.

Key words *Medicago sativa* · Autotetraploid linkage map · Microsatellites SSR · Inbreeding depression · Single dose allele (SDA) analysis

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Introduction

Cultivated alfalfa (*Medicago sativa* ssp. *sativa* L.), the most important forage crop in North America (Barnes et al. 1988) is an autotetraploid (2n=4x=32). However, because of the complexity of the tetrasomic inheritance of alfalfa, all three existing alfalfa genetic linkage maps used diploid (Brummer et al. 1993; Echt et al. 1993; Kiss et al. 1993) rather than tetraploid alfalfa mapping populations. Another reason for mapping in diploid populations is the complexity of analyzing polyploid (especially autopolyploid) linkage relationships and the lack of computer programs to accommodate such data. In the only attempt to map tetraploid alfalfa, linkage relationships were manually calculated, and only one linkage group containing six loci was presented (Yu and Pauls 1993). In other autotetraploid crops such as potato (*Solanum tuberosum* L.), linkage maps have also been constructed at the diploid level (Bonierbale et al. 1988). A method to estimate linkage relationships using single-dose restriction fragments (SDRF) has been developed in sugarcane (*Saccharum spontaneum* L.) (Wu et al. 1992, Da Silva et al. 1993). In this method, alleles that are present in single copies in the parental lines are scored individually based on their presence or absence in the progeny, and linkage relationships are established for each individual chromosome. Homologous linkage groups can then be combined into consensus linkage groups. SDRF analysis was used to map diploid potato (Bonierbale et al. 1988) as well as diploid alfalfa (Echt et al. 1993). SDRF analysis for genomic mapping of tetraploid alfalfa has not yet been reported.

The three existing diploid alfalfa linkage maps were developed using: (1) restriction fragment length polymorphism (RFLP) markers mapped in a cultivated alfalfa on the diploid level (CADL) crossed with *Medicago sativa* spp. *caerulea* (Less. Ex Ledeb.) Schmalh, an uncultivated diploid form of alfalfa (Brummer et al. 1993); (2) RFLP and random amplified polymorphic DNA (RAPD) markers mapped in a backcross population of CADL generated from non-inbred parents (Echt et al. 1993); and (3) RFLP, RAPD, isozyme, and morphological markers mapped in a cross between *Medicago sativa* spp. *caerulea* and *Medicago sativa* spp. *quasifalcata*, another diploid relative of alfalfa (Kiss et al. 1993). All three mapping groups reported a large percentage of loci with distortion from Mendelian segregation: 48%, 34% and 55% (Brummer et al. 1993; Echt et al. 1993, Kiss et al. 1993, respectively).

Simple sequence repeat (SSR) or microsatellite DNA markers are polymerase chain reaction (PCR)-based, multi-allelic, co-dominant genetic markers. In their development, PCR primers are selected to produce one amplification product per genotype in an inbred crop such as soybean, and two amplification products in a heterozygous diploid genotype (Cregan et al. 1994). This eliminates the multiple banding patterns and the resulting complexity which exists with other molecular markers such as RFLP or RAPD. The simplicity of the banding pattern and the multi-allelic nature of SSR loci may be extremely useful in the interpretation of segregation data in an autotetraploid crop such as alfalfa, where up to four alleles per genotype may be observed at any given locus (Diwan et al. 1997). SSR markers are routinely used in the human and other mammalian genetic linkage maps (Murray et al. 1994; Dietrich et al. 1994). In plants, SSRs have been used for the construction of linkage maps in *Arabidopsis thaliana* (Bell and Ecker 1994), and soybean [*Glycine max* (L.) Merr.] (Cregan et al. 1999), and in a recent study, they were found to be abundant in the alfalfa genome (Diwan et al. 1997). That study also indicated the potential use of SSRs in the construction of both diploid and tetraploid alfalfa linkage maps.

The objectives of the study reported here were (1) to integrate a selected group of SSR loci into an existing diploid alfalfa RFLP linkage map; (2) to compare levels of segregation distortion between diploid and tetraploid alfalfa; and (3) to determine if the simplicity and highly multi-allelic characteristics of SSR markers together with the use of the SDRF method would facilitate mapping in tetraploid alfalfa.

Materials and methods

Development of simple sequence repeat markers

Selection of SSR-containing sequences from Genbank and from a genomic alfalfa DNA library, as well as selection and preparation of PCR primers to microsatellite loci have been described elsewhere (Diwan et al. 1997).

Alfalfa genetic material and DNA isolation

The diploid F_2 mapping population of 86 plants resulted from a cross between a diploid alfalfa plant (W2xiso no. 3) and a *M. sativa* spp. *caerulea* plant (PI 440501 no. 2). This mapping population has been described elsewhere (Brummer et al. 1993).

The tetraploid F_2 population consisted of 57 plants. Two alfalfa (*Medicago sativa* L.) plants – MN White-MA (MA-4), a plant with white flowers, and A-620, a plant with purple flowers – were crossed at Athens, Georgia to produce an F_1 plant. MA-4 is a cytoplasmic male-sterile (CMS) maintainer selected from a Turkistan germplasm and was developed from a cooperative breeding program between USDA-ARS St. Paul, Minnesota and The Institute of Plant Breeding and Acclimatization, Radzikow, Poland. MA-4 was obtained from Dr. Donald Barnes USDA-ARS, and the Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minnesota. A-620 is a selection from the var. Apollo and was obtained from Sue Croughan, LSU, Rice Research Station, Crowley, Louisiana. The F_1 plant was selfed to produce the F_2 population.

A sample of DNA from each F_2 plant was obtained from leaf tissue. DNA was isolated using a standard CTAB (hexadecylatrimethylammonium bromide) procedure (Brummer et al. 1991).

Polymerase chain reaction conditions and PCR product separation

The 5' end primer from each of the PCR primer sets, except for MTLEC2 A and Afat15, which contain $(AT)_n$ core motifs, was 5' end-labeled using 10 pmoles of the primer, 5 µl 10×T4 polynucleotide kinase buffer [0.5 *M* Tris-HCl, pH 7.6, 100 m*M* $MgCl_2$, and 100 m*M* 2-mercaptoethanol (United States Biochemical, Cleveland, Ohio)], 20 pmole [γ-[^{32}P]ATP at 110 Bq/mmol, and 3 U of T4 polynucleotide kinase (United States Biochemical, Cleveland, Ohio). Reaction mixes contained 30 ng of genomic DNA, 1.5 m*M* Mg^{2+}, 0.15 µ*M* of 3' and 5´ end-labeled primers, 200 µ*M* of each dNTP, 1×PCR Buffer (50 m*M* KCl, 10 m*M* Tris-HCl, pH 9.0, 0.1% Triton X-100), and 1 U *Taq* DNA polymerase in a total volume of 10°µl. For the MTLEC2 A and Afat15 loci, unlabeled 3' and 5' end primers and the addition of 0.1 µl of 110 Bq/mmol α-[^{32}P]dATP (10 FCi/µl)] substituted for the end-labeled primers. Thermal cycling consisted of a 30-s denaturation at 94°C, 25-s annealing at 51° or 52°C, and 25-s extension at 68°C for 32 cycles on a MJ Research model PTC-100 thermocycler (MJ Research, Watertown, Mass.). PCR products (3 µl/lane) were separated on a standard DNA sequencing gel containing 6% polyacrylamide, 8 *M* urea, and 1×TBE, at 60 W constant power for 2–3 h at Beltsville, Md.

Allele naming

In the tetraploid population, allele names were a combination of the locus name followed by the letters A, B, C, or D, where A is the highest molecular-weight allele in the F_1 plant, B has the second highest molecular weight, etc. In the diploid population, the letters a and b in the allele names correspond to their parental origin: a – W2xiso no. 3, and b – *M. sativa* spp. *caerulea*.

Genetic mapping

In an initial evaluation, each primer set was tested on the parents and F_1 plant of the diploid mapping population. Those primer pairs that produced two products of predicted size in the F_1 plant, one of which was present in each parent, were used to determine the genotype of each F_2 plant in the mapping population and were then tested on the parents and F_1 plant of the tetraploid mapping population.

Ten SSR loci (Table 1) were selected to characterize the diploid and tetraploid segregating populations. Goodness of fit to a 1:2:1 model was tested for each of the loci of the diploid popula-

Table 1 Sequences of SSR (microsatellite) alfalfa PCR primer pairs, allele size range and number for each locus in both the diploid and tetraploid mapping populations. All primer sequences are given in the 5' to 3' direction

Locus	Core motif	5´ end primer (sense)	3´ end primer (anti-sense)	Allele size range (bp)	Number of alleles
AFat15	$(AT)_{23}$	ttacgggtctagattagagagtatag	caaaatgagtatagggagtgg	150–259	7
AFca1	$(CT)_4(CA)_{10}$	cgtatcaatatcgggcag	tgttatcagagagagaaagcg	112–131	7
AFca11	$(CA)_{11}$	cttgagggaactattgttgagt	aacgtttcccaaaacatactt	136–160	6
AFca16	$(CA)_{12}$	ggtcgaaccaagcatgt	taaaaaacattacatgacctcaaa	87–101	7
AFct11	$(CT)_{12}$	ggacagagcaaaagaacaat	ttgtgtggaaagaataggaa	178–194	3
AFct32	$(CT)_{14}$	tttttgtcccacctcattag	ttggttagattcaaagggttac	101–157	9
AFct45	$(CT)_8AT(CT)_3$	taaaaaacggaaagagttggttag	gccatcttttcttttgcttc	123–145	5
AFct60	$(CT)_{21}$	cctccctaactttccaaca	tggatcaacgtgtctttca	130–171	6
AFctt1	$(CTT)_9(CAA)_3$	cccatcatcaacattttca	ttgtggattggaacgagt	99–126	10
MTLEC2A	$(AT)_{19}$	cggaaagattcttgaatagatg	tggttcgctgttctcatg	181–191	4

tion. In the tetraploid population, ratios of 1:3, 1:8:8:18:1, and 1:1:1:1:1:1:6:6:6:6:6 were tested for loci with two, three and four segregating alleles, respectively. The MAPMAKER 3.0b program (Lander et al. 1987) was used to order the ten SSR loci along with the 108 previously mapped RFLP markers in the diploid population (Brummer et al. 1993). All pairs of loci with a map distance of less than or equal to 40 cM and a LOD score exceeding 3.0 were considered to be linked.

The SDRF method (Wu et al. 1992; Da Silva et al. 1993) was adjusted in this study to use with SSR loci, and will be referred to as the single dose allele (SDA) analysis. SDA analysis was used to construct a linkage map of diploid alfalfa using the same ten SSR and 108 RFLP marker data used in the MAPMAKER 3.0b analysis described above. This allowed a comparison of linkage relationships between maps that were derived with SDA versus co-dominant alleles. The female and male parent derived data were analyzed separately using MAPMAKER 3.0b (Lander et al. 1987) (maximum distance=40 cM; LOD=3). SDA also was used to construct a tetraploid alfalfa linkage map using separate male and female derived data from the ten SSR loci and MAPMAKER 3.0b (Lander et al. 1987) (maximum distance=40 cM; LOD=3).

Using the SDA analysis, we scored alleles present in single copies in the F_1 plant individually for presence or absence in the F_2 progeny. In an attempt to verify if alleles were present in simplex, we expected a single-dose allele to segregate in a 3:1 ratio (present:absent) in the F_2 tetraploid mapping population. For an allele to be considered usable for mapping at the tetraploid level, it had to meet two criteria. (1) Its origin from one parent or the other should be obvious; i.e., it must either be present in one parent and absent in the other, or if the allele is present in both parents, its origin could be unequivocally determined by elimination. For example, at the Afct32 locus (Fig. 1B) alleles A (126 bp) and B (120 bp) present in the F_1 plant and in the female (MA-4) parent were clearly inherited from the female parent. Since each parent can contribute up to two alleles, allele D (102 bp), which is present, in both parents must therefore have been inherited from the male (A-620) parent. (2) The allele must segregate 3:1 in the F_2. Chi-square tests were used to determine goodness of fit of the segregation ratios. At the diploid level, all alleles were considered single-dose; therefore, all were usable for mapping.

Results and discussion

Genotypes and phenotypes of SSR loci of the diploid and tetraploid mapping populations

Each of the PCR primer sets to MTLEC2 A and to nine selected SSR loci isolated from the genomic library (Table 1) produced up to two bands per genotype in the diploid plants, and two to four bands in each of the tetraploids. The number of alleles per locus in both diploid and tetraploid populations ranged from 3 to 10 at the Afct11 and Afctt1 loci, respectively. Genotypes of the parental lines, the F_1 progeny, and the F_2 plants of the diploid mapping population could be determined unambiguously (Fig. 1, panels A and C). The unambiguous detection of genotypes has been previously shown in other crops such as soybean (Akkaya et al. 1992). In the tetraploid population, the determination of the genotype of the F_1 plant based on the parental genotypes was usually easy. However, the genotypes of the F_2 tetraploid plants were not obvious for either triallelic (AABC vs. ABBC vs. ABCC) or for diallelic genotypes that were in triplex (AAAB vs. ABBB). Also, it was difficult to distinguish duplex versus triplex (AABB vs. AAAB vs. ABBB) conditions because in some instances within a lane, bands which contained double- or triple-dose alleles appeared darker than bands which represented a single-dose allele (e.g. Fig. 1B, lanes 6, 7), while in other cases all bands had the same intensity regardless of allele dosage (Fig. 1B, lanes 1, 5). Therefore, segregation analysis in the tetraploid mapping population was performed based on the phenotype rather than the genotype of the F_2 plants (e.g., genotypes AABC, ABBC, and ABCC all have the phenotype ABC). The triallelic condition was the most common in the tetraploid population with seven of the ten loci having 3 segregating alleles, two loci (AFat15 and AFca16) had 2 alleles, and one locus (AFct32) had 4 segregating alleles (Fig. 1B).

Segregation distortion

Chi-square tests indicated segregation distortion in both diploid and tetraploid populations (Table 2). In the diploid population, three of the four loci which deviated significantly from Mendelian expectation, AFca1, AFctt1, and AFct45, had a higher proportion than expected of heterozygous F_2 progeny (85%, 83% and 85% heterozygous, respectively). The fourth locus, AFat15, had more than the expected homozygous genotypes with the W2xiso no. 3 parental allele and fewer than expected homozygous genotypes with the PI 440501 parental allele. In the tetraploid population, three loci showed segrega-

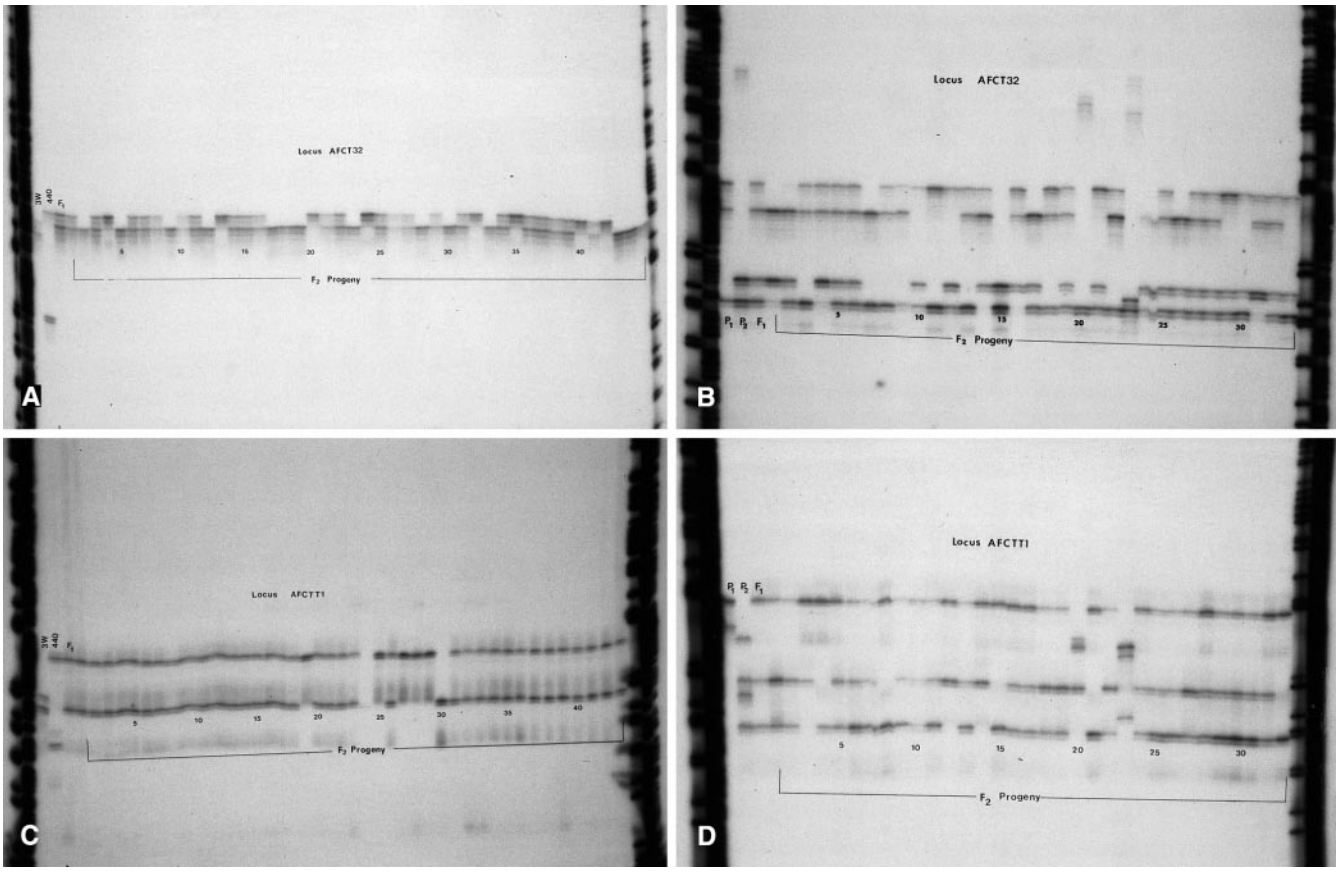

Fig. 1A–D Segregating SSR loci of diploid and tetraploid alfalfa F_2 mapping populations. **Panels A** and **B** show locus AFct32 in the diploid and tetraploid mapping populations, respectively. Allele sizes are as follows: **Panel A**, *3 W* 137 bp and 141 bp, *440* 115 bp and 146 bp, F_1 141 bp and 146 bp; **Panel B** P_1 101 bp, 120 bp, and 126 bp, P_2 101 bp, 106 bp, and 157 bp, F_1 101 bp, 106 bp, 120 bp, and 126 bp (alleles AFct32D, AFct32 C, AFct32B and AFct32 A, respectively). **Panels C** and **D** show locus AFctt1 in the diploid and tetraploid mapping populations, respectively. Allele sizes are as follows: **Panel C**, *3 W* 108 bp and 112 bp; *440* 99 bp and 122 bp, F_1 108 bp and 122 bp. **Panel D** P_1 120 bp and 126 bp, P_2 100 bp, 106 bp, 109 bp, and 118 bp, F_1 100 bp, 109 bp, and 126 bp (alleles AFctt1 C, AFctt1B and AFctt1 A, respectively)

tion distortion. However, except for locus AFat15, loci with segregation distortion were different at the two-ploidy levels, indicating that distortion is present in different areas of the genome in the two populations. As with the diploid population, loci in the tetraploid population usually had more heterozygous (tri- or tetra-allelic) genotypes than expected. An excess of heterozygous genotypes supports the theory of maximum heterozygosity in alfalfa (Dunbier and Bingham 1974). This theory emphasizes the importance of intra-locus interactions in autotetraploids and associates tri- and tetra-allelic loci with maximum performance, as measured by forage yield fertility and seed weight. Maximum heterozygosity was also found to positively affect potato (*Solanum tuberosum*) tuber yield (Bonierbale et al. 1993).

Table 2 Chi-square tests to determine deviation from Mendelian segregation in the diploid and tetraploid alfalfa mapping populations

Locus	Mapping populations[a]	
	Diploid[b] χ^2	Tetraploid[c] χ^2
AFat15	6.0*	11.5***
AFca1	40.8***	2.9
AFca11	4.2	47.1***
AFca16	3.7	0.2
AFct11	1.4	5.6
AFct32	0.2	5.1
AFct45	41.7***	7.6
AFct60	1.2	1.3
AFctt1	36.3***	7.5
MTLEC2A	0.7	21.9***

*, *** Significant at P=0.05 and 0.001, respectively

[a] Mapping population size is 86 and 57 F_2 plants for diploid and tetraploid alfalfa, respectively

[b] Loci at the diploid level were tested for a 1:2:1 segregation ratio

[c] All loci at the tetraploid level except for AFat15, AFca16 and AFct32 had three segregating alleles and were tested for a 1:8:8:18:1 segregation ratio. AFat15 and AFca16 with two and AFct32 with four segregating alleles, respectively, were tested for a 3:1 and 1:1:1:1:1:1:6:6:6:6:6 segregation ratio, respectively

Mapping in the diploid using co-dominant marker data

Using the 108 RFLP loci, Brummer et al. (1993) reported ten linkage groups with a total map length of 467.5 cM. The addition of the ten SSR markers length-

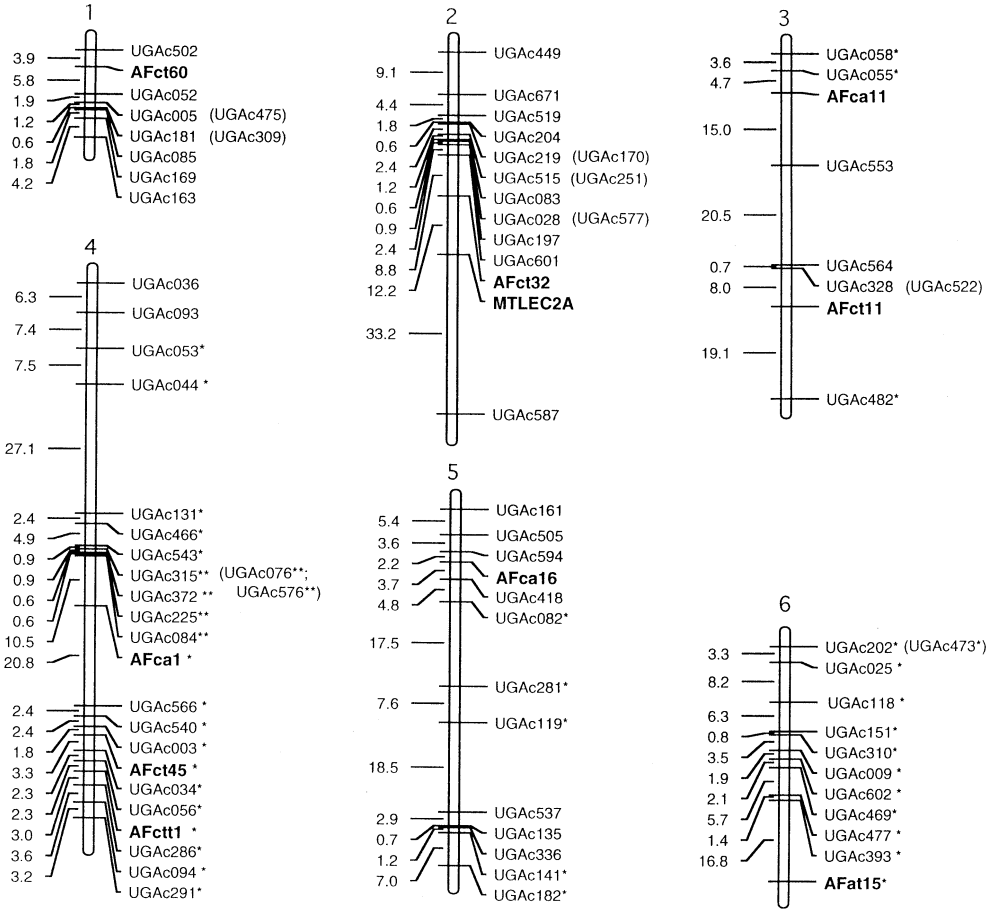

Fig. 2 RFLP linkage groups of diploid alfalfa (Brummer et al. 1993), which contain SSR markers. *Single asterisk* indicates loci with segregation distortion ($P<0.05$); *two asterisks* indicate clones for which more than 90% of the F_2 progeny were heterozygous

ens the map to 534.6 cM. The ten SSR loci appeared to be randomly distributed across the diploid alfalfa genome and mapped to six of the original ten linkage groups in the diploid alfalfa RFLP map (Fig. 2) (Brummer et al. 1993). These findings are similar to soybean where 40 SSR markers were shown to be distributed randomly in a linkage map which also included 118 RFLP and RAPD, 13 morphological and pigmentation, and seven isozyme markers (Akkaya et al. 1995). Two groups of SSR markers, AFct32 and MTLEC2 A in linkage group 2 and AFctt1, AFct45 and AFca1 on linkage group 4, were linked (Fig. 2). AFctt1, AFct45, and AFca1 are the three loci with segregation distortion resulting from a greater than expected number of heterozygous F_2 progeny. The RFLP loci in this linkage group also had an excess of heterozygotes, including 7 RFLP loci at which 90% or more of the F_2 progeny are heterozygous (Brummer et al. 1993). AFat15, the locus which had more than the expected homozygous genotypes with the W2xiso no. 3 allele, mapped to linkage group 9 where all other loci showed the same segregation distortion resulting from an excess of progeny homozygous for the W2xiso no. 3 allele. It is apparent that there are two types of segregation distortion in the diploid population. In one type, both homozygotes are deficient in number and heterozygotes are in excess (linkage group 4), and in the second only one homozygote is deficient and the heterozygotes are in excess (linkage group 9). A simple explanation is the presence of 1 deleterious recessive allele in one case (linkage group 9), or 2 such alleles that were contributed by both parental lines (linkage group 4). This is supported by loci within each linkage group showing the same type of segregation distortion.

SDA analysis of the diploid mapping population

Using SDA to determine linkage relationships among the ten SSR and 108 RFLP loci at the diploid level resulted in six pairs of homologous linkage groups corresponding to six of the ten linkage groups of the co-dominant diploid map (Brummer et al. 1993, Fig. 2). The conserved linkage groups are numbers 1, 2, 3, 6, 7, and 10. A typical example of a conserved linkage group (group 2) is presented in Fig. 3A. The homologous female- and male-derived maps are quite similar to the map derived from co-dominant data. However, there were some rearrangements of loci within the SDA linkage groups (Fig. 3A) as compared to the co-dominant map. Loci on linkage group 5 of the co-dominant marker-derived map were placed in two separate linkage groups when SDAs from the Wxiso no. 3 (CADL) parent were analyzed (Fig. 3, panel C). In contrast, loci in the homologous linkage group from the PI 440501 no. 2 (*M. sativa* spp. *caerulea*)

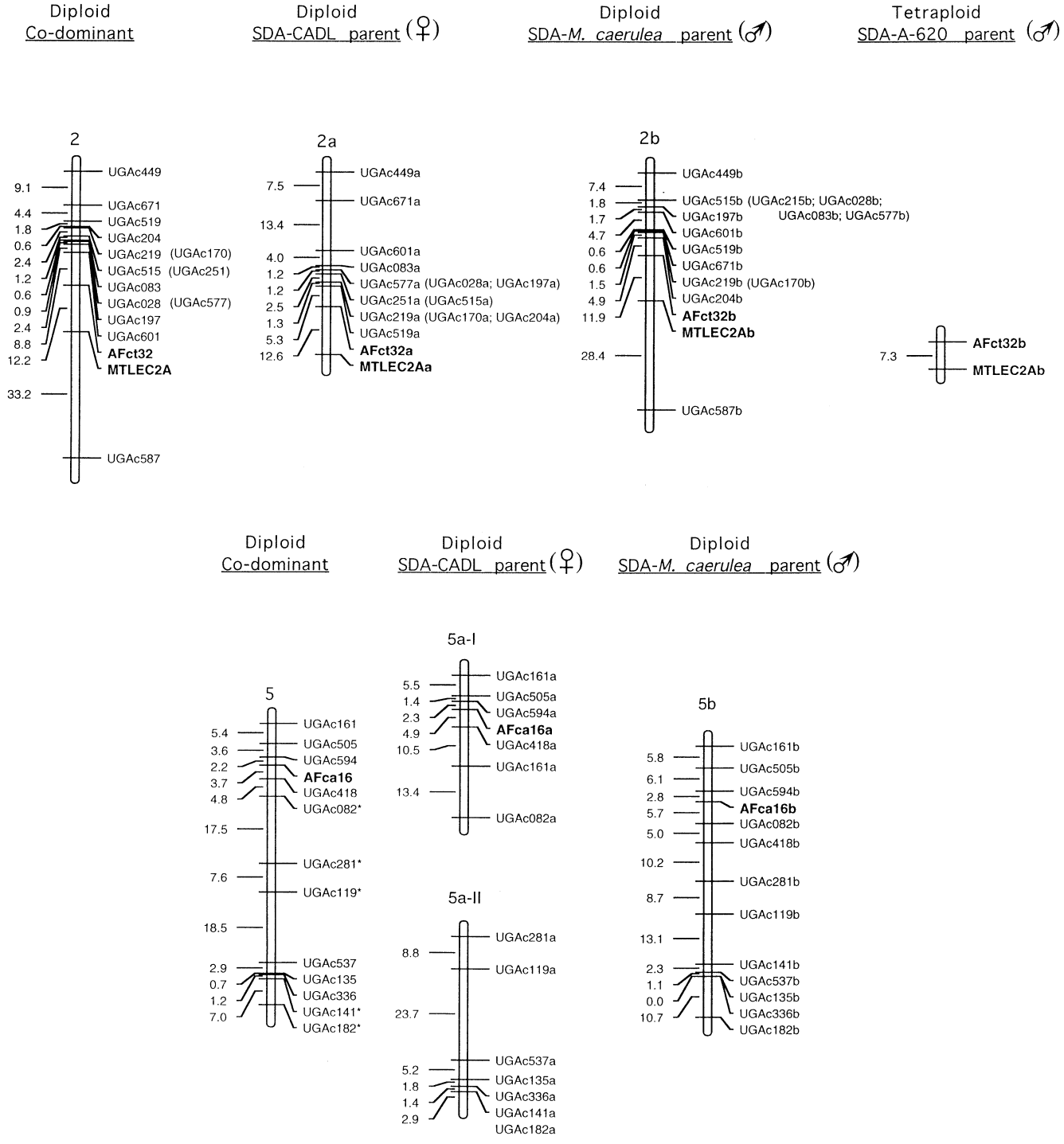

Fig. 3A–C RFLP and SSR linkage groups of diploid alfalfa, and SSR linkage groups of tetraploid alfalfa constructed by the single-dose allele method, which are homologous to linkage groups 2 (**Panel A**), 4 (**Panel B**), and 5 (**Panel C**) of the co-dominant derived diploid alfalfa linkage map (Brummer et al. 1993). Allele names in the diploid map followed by the *letters a* or *b* originated from the W2xiso no. 3 or PI 440501 no. 2 parent, respectively

parent remained in one linkage group. Thus, in seven of ten instances, the linkage groups produced by the SDA analysis had similar linkage associations to these derived from the co-dominant data. The three exceptions were linkage groups 4, 8, and 9. Although some of the original linkage blocks of these linkage groups remained intact, other linkage blocks moved from linkage groups 8 and 9 to linkage group 4. These rearrangements were not random and were associated with the parental origin of the alleles, i.e., alleles from linkage 8 of the co-dominant derived map that originated from the CADL (female) par-

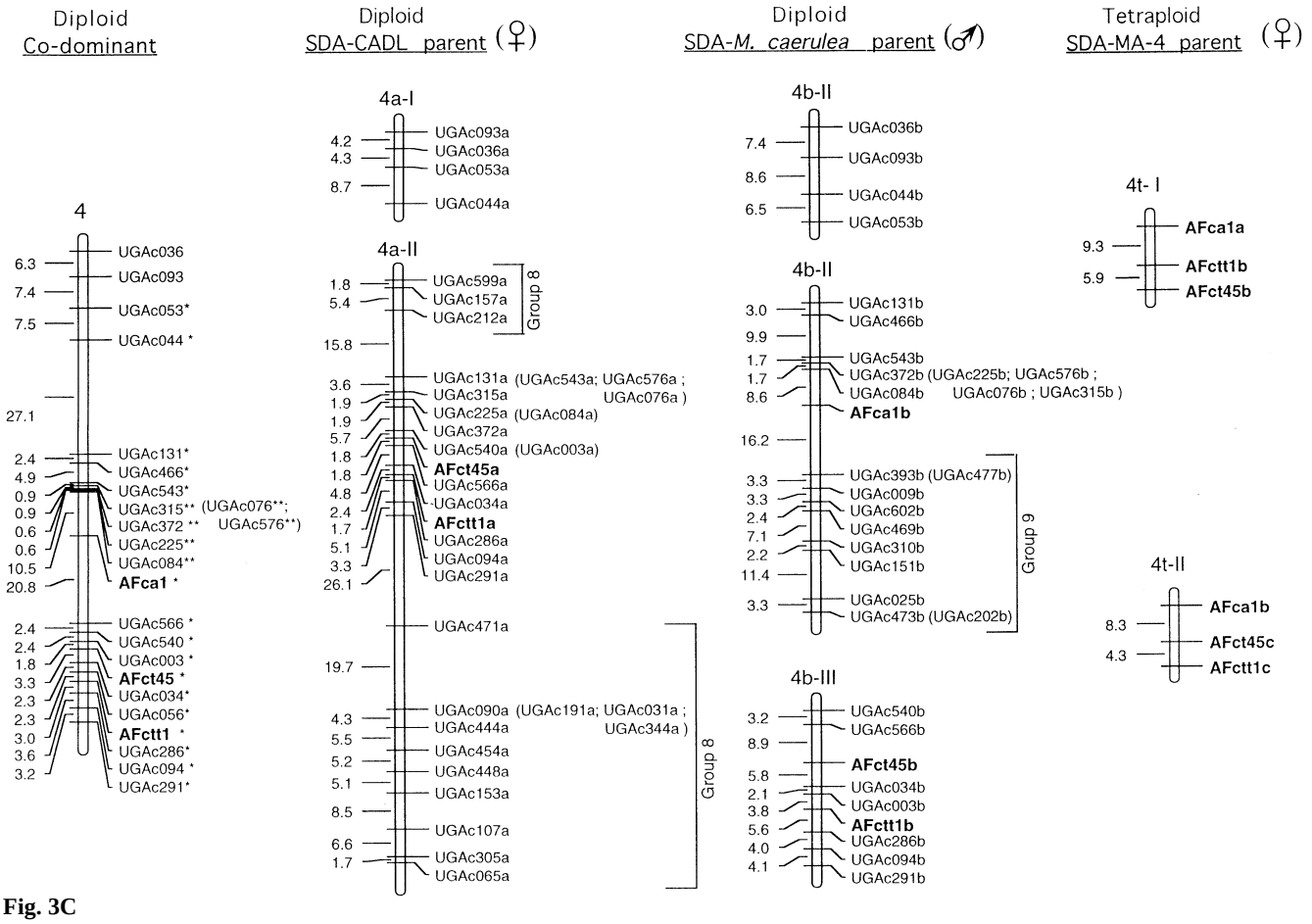

Fig. 3C

ent moved to the SDA linkage groups 4 of that parent (Fig. 3, panel B), while alleles from linkage group 9 of the co-dominant derived map that originated from the *M. caerulea* (male) parent moved to the SDA linkage group 4 of the male parent. These rearrangements in linkage group 4 are probably due to the severe segregation distortion in this group where 92% of the loci have an excess of heterozygotes among the F_2 progeny. In general, segregation distortion was present in each case where the SDA-derived linkage groups differ from their co-dominant derived homologs. Furthermore, when using SDA analysis, each locus is mapped with only half of the information that is available for mapping co-dominant loci. Consequently, this is likely to bias determination of linkage groupings.

SDA analysis of the tetraploid mapping population

Eighteen alleles from nine of the ten SSR loci met the criteria for single-dose (simplex) alleles in the tetraploid population (Table 3). There may be two reasons why some alleles did not meet these criteria: (1) alleles were not in simplex in the F_1, and (2) some form of selection resulted in the elimination of particular genotypes. Although, one of the alleles, Afct45C, showed segregation distortion, it was included in the mapping data. Of the 18 alleles 10 were associated with loci that were unlinked; the other 8 alleles were associated with loci that formed three linkage groups (Fig. 3, panels A, B). Because of the relatively small number of alleles available for mapping loci in the tetraploid population, compari-

Table 3 Single-dose alleles found in the tetraploid mapping population – the number of F_2 progeny in which the allele was present or absent, and the chi-square tests for 3:1 (present/ absent) ratio

Allele	Present	Absent	χ^2
Afca1A	43	13	0.09
AFca1B	48	8	3.43
AFca11B	41	13	0.03
AFca11C	46	8	2.99
AFca16A	41	12	0.16
AFct11A	47	10	1.69
AFct11B	46	11	1.00
AFct32A	45	12	0.55
AFct32B	45	12	0.55
AFct32C	39	18	1.32
AFct32D	45	12	0.55
AFct45B	48	9	2.57
AFct45C	52	5	8.00
AFct60A	44	13	0.14
AFct60B	46	11	0.99
AFctt1B	45	12	0.55
AFctt1C	49	8	3.65
MTLEC2AB	44	12	0.39

son of the diploid to the tetraploid SDA map was difficult. However, two of the tetraploid linkage groups were homologous and similar to linkage group 4 of the diploid linkage map. The order of the loci was different in these two homologous tetraploid linkage groups. This could be attributed to the significant genotypic segregation distortion that was associated with Afct45C. The third linkage group was homologous to linkage group 2 of the diploid map. As might be anticipated, map distances were different between the diploid and tetraploid linkage maps.

Conclusions

Alfalfa is an outcrossing species that suffers from inbreeding depression (Busbice and Wilsie 1966). Inbreeding depression has been hypothesized to result from either deleterious recessive alleles that result in distorted genotypic segregation, or reduced heterozygosity per se. Both populations used in the current study were inbred one generation. In addition, the diploidization process used to produce the CADL parent of the diploid mapping population further reduced the number of heterozygous loci in a manner similar to one generation of inbreeding. Thus, in the populations used here, and particularly in the diploid population, linkage relationships may be biased. Additionally, segregation distortion probably resulted in inaccurate linkage groupings when the SDA analysis was used for mapping in the diploid population because SDA is more sensitive to segregation distortion (linkage group 4) than the co-dominant mapping method. In the current study, segregation distortion in the F_2 tetraploid population was only slightly less than that of the diploid (three loci with segregation distortion vs. four) and was higher than previously found in a tetraploid F_1 mapping population (Yu and Pauls 1993), where only 2 RAPD loci out of 32 did not fit Mendelian expectations. This suggests that mapping in alfalfa should be conducted in an out-crossed (F_1) tetraploid population.

Yu and Pauls (1993) studied strategies for molecular mapping in an F_1 population of tetraploid alfalfa using RAPD markers and concluded that mapping in tetraploid alfalfa is feasible. However, because of the dominant nature of RAPD markers, only markers that were in the simplex (Mmmm) or duplex (MMmm) condition in the parental lines were expected to segregate in the F_1 progeny and be useful for mapping. Simple sequence repeat markers which are co-dominant and multi-allelic provide a much more efficient marker system than RAPDs for the construction of a tetraploid map. The single-dose allele analysis which was adapted from RFLP mapping studies (Wu et al. 1992; Da Silva et al. 1993) and used here to map the SSR loci, coupled with the high abundance, ease of following segregation, and multi-allelic and co-dominant nature of SSRs, provides a useful tool for mapping of tetraploid alfalfa as well as other polyploid crops.

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