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ORIGINAL PAPER

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Investigation of genomic organization in switchgrass (*Panicum virgatum* L.) using DNA markers

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Abstract We report an early investigation into genomic organization and chromosomal transmission in switchgrass based on restriction fragment length polymorphism (RFLP) markers. The segregation of 224 single dose restriction fragments (SDRF) in 85 full-sib progeny of a cross between the genotypes Alamo (AP13) and Summer (VS16) was used to determine linkage associations in each parent. In the seed parent AP13, 11 cosegregation groups were identified by 45 SDRF markers with a cumulative recombination length of 412.4 cM. In the pollen parent VS16, 57 SDRF markers were assigned to 16 cosegregation groups covering a length of 466.5 cM. SDRF markers identified by the same probes and mapping to different cosegregation groups were used to combine the two maps and identify homology groups. Eight homology groups were identified among the nine haploid linkage groups expected in switchgrass. The high incidence of repulsion phase associations indicates that preferential pairing between homologous chromosomes is predominant in switchgrass. Based on marker distribution in the paternal map (VS16), we estimated the recombinational length of switchgrass genome to be 4,617 cM. In order to link 95% of the genome to a marker at a 15-cM distance, a minimum of 459 markers will be required. Using information from the ratio of repulsion to coupling linkages, we infer that switchgrass is an autotetraploid with a high degree of preferential pairing. The information presented in this study establishes a foundation for extending genetic mapping in this crop and constitutes a framework for basic and applied genetic studies.

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Introduction

Switchgrass (*Panicum virgatum* L.), a warm season, C_4 perennial grass, is native to most of North America (Hitchcock 1971). It has been widely grown for summer grazing and soil conservation and was chosen by the Bioenergy Feedstock Development Program (BFDP) at the U.S. Department of Energy as a model bioenergy species from which renewable sources of transportation fuel or biomass-generated electricity could be derived (Sanderson et al. 1996).

Switchgrass is largely cross-pollinated (Talbert et al. 1983) and self-incompatible, possibly under gametophytic control similar to the S-Z system found in other members of the Poaceae (Martinez-Reyna and Vogel 2002). Natural populations are broadly classified into two main ecotypes, lowland and upland, based on morphology and natural habitat (Porter 1966). That cytological differences exist between the two major ecotypes was confirmed by Hultquist et al. (1996). Several different chromosome numbers and ploidy levels have been reported for switchgrass; these range from $2n = 18$ to $2n = 36$, 54, 72, 90, and 108 (Church 1940; Burton 1942; Nielson 1944). The most recent polyploidization events that established modern switchgrass lineages are estimated to have occurred less than two million years (Huang et al. 2003). Hybridization between the two cytotypes is possible only between plants of a similar

ploidy level (Martinez-Reyna et al. 2001). Intermating between octaploid and tetraploid populations is believed to be prevented by post-fertilization processes that inhibit normal seed development, similar to endosperm incompatibility caused by the endosperm balance number system found in other species (Martinez-Reyna and Vogel 2002).

To date, switchgrass has not received much attention in genetic research. Genetic mapping will enable switchgrass breeders to more quickly and cost-effectively identify chromosomal regions and monitor their inheritance through generations. The development of linkage maps have made possible the dissection and tagging of many economically important traits in many crops. The information provided by the genetic linkage map is exploited to correlate molecular markers with a phenotype in a segregating population, thereby presenting a great potential for marker-assisted plant breeding and the deployment of favorable gene combinations (Ribaut and Hoisington 1998).

The construction of linkage maps in polyploid species is more complicated than in diploids because of the higher number of alleles and the greater number of possible genotype combinations (Sorrells 1992). In many species, the genotypes are not easy to identify based on their phenotypes, and the genomic constitution of the polyploid is uncertain (Wu et al. 2001). In allopolyploid species, such as wheat (*Triticum aestivum*), meiotic pairing occurs predominantly between homologous chromosomes. Thus, their genetics is similar to that of diploids except for the multiple genomes, and linkage mapping in these species applies the same statistical procedures established for estimating recombination in diploid species (Lander and Green 1987). In polyploid species that have not been well-characterized, genetic mapping is further complicated by factors such as preferential pairing between homologous chromosomes and double reduction that leads to distortion of the segregation ratios needed to estimate recombination fractions (Wu et al. 2002).

Several genetic models for linkage analysis in polyploids have been suggested. Most of these models are aimed at the application of codominant molecular markers in full-sib families based on the assumptions of bivalent, multivalent pairing, or both (Wu et al. 2001, 2002; Luo et al. 2002). To simplify linkage analysis in polyploids, Wu et al. (1992) designed a method for mapping polyploids based on the segregation of single-dose restriction fragments (SDRF) that segregate in a ratio of 1:1 (absence versus presence) in the progeny. Single-dose loci are equivalent to simplex alleles in autoploids or heterozygous alleles in diploid genomes of alloploids. The first step in the construction of a genetic map using this method is to determine the dosage of each marker locus based on its segregation ratio using a Chi-squared test. Marker loci present in single doses are ordered in a framework map for individual chromosomes, while fragments present in higher dosage are used to order the individual linkage groups into homologous groups and for the indirect detection of SDRFs linked in repulsion (Da Silva and Sorrells 1996). The SDRF mapping procedure has been applied successfully in constructing linkage maps in sugarcane (*Saccharum officinarum*; Da Silva et al. 1993), sour cherry (*Prunus cerasus*; Wang et al. 1998), potato (Li et al. 1998) and alfalfa (Brouwer and Osborn 1999).

The purpose of the investigation reported here was to investigate the genomic organization and chromosomal transmission in switchgrass. The inheritance, segregation, and linkage between heterologous restriction fragment length polymorphic (RFLP) markers that have been mapped in other grass species were examined in two tetraploid ($2n=4$, $x=36$) switchgrass cytotypes and used to estimate the recombinational length of the switchgrass genome and the degree of preferential chromosome pairing and to construct the first low-density linkage map of this crop.

Materials and methods

Mapping population

The mapping population of switchgrass (*Panicum virgatum* L.) consisted of a full-sib family of 85 individuals derived from a cross between two outbred parents, specifically an upland tetraploid and a lowland tetraploid genotype that showed extensive genetic divergence based on an RFLP survey of 21 accessions (Missaoui 2003). The lowland genotype, Alamo (AP13), was used as the seed parent and the upland genotype Summer (VS16) was used as the pollen parent. The hybrid progeny has an intermediate phenotype between the two parents. The true hybrids have a triangular patch of hair on the upper side, near the base of the leaves, which is absent in the maternal plant. This phenotype was used to screen against individuals derived from self-pollination. The hybrid progeny were also tested for accidental selfing using RFLP markers. Fully expanded leaves were collected from each plant. Leaf samples were freeze-dried and powdered in a Tecator (Foss, Denmark) cyclotec sample mill and stored frozen at −80°C.

DNA extraction and RFLP analysis

Total genomic DNA was extracted from lyophilized tissue using the CTAB method (Murray and Thompson 1982) with slight modifications. The samples were extracted in a buffer containing 5% CTAB, 0.7 *M* NaCl, 10 m *M* EDTA pH 8.0, 50 m *M* Tris-HCl pH 8.0, and 0.1% 2-mercaptoethanol and incubated for 2 h at 65°C with occasional gentle mixing.

Approximately 10 μg of DNA from each individual were digested with one of four restriction enzymes, *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I, that showed polymorphism between the parents. The digested product was electrophoresed on 0.8% agarose gels using 1× NEB

buffer. The DNA was then transferred by capillarity to a Hybond N+ nylon membrane (Amersham, Arlington Heights, Ill.) in accordance with the technique of Southern (1975). Probes were labeled using the random primer labeling method (Feinberg and Vogelstein 1983). DNA filters were pre-hybridized in hybridization buffer [6× SSPE pH 7.0, 5× Denhardt Solution, and 0.5% sodium dodecyl sulfate (SDS)] containing 200 mg ml^{-1} of denatured Herring sperm DNA at 65°C for 4–6 h. This was followed by the addition of the labeled probe into the pre-hybridization mix, and overnight hybridization at 65°C. After hybridization, the filters were washed for 30 min with the following buffers, 2× SSC, 0.1% SDS, 1× SSC, 0.1% SDS, at 65°C, and exposed to X-ray film.

A total of 389 heterologous grass probes from four sources were used for the detection of polymorphism between the parents. The DNA probes mapped were 74 rice (*Oryza sativa*) cDNA probes (prefix: RZ; Causse et al. 1994), 17 Bermuda grass (*Cynodon dactylon*) hypomethylated (*Pst*I) genomic clones (prefixes: pCD and T574), and eight cDNA clones from *Pennisetum* apomictic pistils (prefix: pPAP).

Linkage analysis and mapping of markers

RFLP phenotypes were scored manually from autoradiographs. The segregation of each scorable band was treated independently based on its presence or absence in the progeny (Fig. 2). Plants containing alleles from the seed parent were scored as '4' or '1', for presence or absence, respectively; plants containing alleles from the pollen parent were scored as '5' (present) or '3' (absent). Ambiguous bands were designated as '0'. Multiple loci detected by the same probe were assigned a letter after the probe designation. Loci differing between the parents and segregating in the progeny were tested for goodness-of-fit to the theoretical ratio of 1:1 using a Chi-squared test. This segregation pattern is characteristic of a SDRF or simplex marker, which is a fragment present in a single copy in the parent and which segregates in a single-dose ratio in the progeny of a cross between two outcrossing parents (Wu et al. 1992). Loci that did not fit a 1:1 ratio were tested for fit to the 5:1 ratio characteristic of tetrasomic inheritance of double-dose restriction fragments (DDRF). Loci that did not fit either of these two ratios at $P = 0.05$ were considered to be single dose if the absolute ratio of present to absent was below 2.24:1, which gives equal χ^2 for simplex and duplex ratios (Mather 1957). Markers present in both parents and segregating in the progeny were tested for fit to the 3:1 (presence:absence) ratio characteristic of a simplex-by-simplex cross. Marker loci that were produced by the same probe and displayed the same segregation pattern were considered to be redundant, and only one was retained for analysis.

The linkage relationships between simplex markers were determined using the computer program MAPMAKER 3.0 (Lander et al. 1987). A separate map was constructed for each parent and the SDRFs were analyzed as an "F_2 backcross". SDRFs were first assigned to linkage groups using two-point analysis at a LOD score of 5 and a maximum recombination fraction of 0.25. This high threshold was chosen to minimize false linkages. Linkage was also tested by reducing the LOD score to 3 to see if more markers could be added to the associated markers. Orders within each group were determined by the COMPARE function of MAPMAKER and the most-likely order selected. The RIPPLE command was then used to verify the order. Loci were sorted according to this order, and double-crossover events indicated by the ERROR DETECT ON option were rechecked for scoring errors on the original autoradiographs. Recombination fractions were converted to centiMorgan (cM) distances using the Kosambi function (Kosambi 1944).

Cosegregation groups were assigned into homology groups based on common markers detected by the same probe on two or more groups. Chromosome pairing behavior was investigated using repulsion linkage between linked and unlinked simplex markers detected by the same probe as well as between markers borne on putative homologous cosegregation groups.

The approximate number of centiMorgans in the switchgrass genome was estimated using the method-of-moment estimator (Hulbert et al. 1988) as modified in method 3 of Chakravarti et al. (1991):

$$E(G) = [n(n-1)2d]/2k,$$

where $E(G)$ is the estimated genome length, n is the number of informative markers, d is the largest observed distance between the locus pairs at a specified LOD score Z, and k is the number of pairs of markers linked at the specified LOD Z or greater. The values used for Z in the estimate were 3, 4, and 5. The values of d and k were obtained directly from the output list of values generated by the LOD function of MAPMAKER.

Expected genome coverage EC_n) was calculated using the method of Bishop et al. (1983):

$$E(C_n) = 1 - P_{1,n};$$

and

$$P_{1,n} = (2R/n+1) * \left[(1-d/2G)^{n+1} - (1-d/G)^{n+1}\right] + (1-Rd/G)(1=d/G)^n$$

where R is the number of chromosomes, d is the maximum distance used to detect linkage at a LOD score of 3, and G is the estimated recombinational genome length in centiMorgans. The minimum number of randomly distributed markers (n) required to cover a proportion (P) of a genome of size (L) at a maximum distance ($2d$) between markers was estimated using the method of Lange and Boehnke (1982) as follows:

$$n = [\log(1-P)]/\log(1-2d)$$

Results

Segregation analysis

A total of 389 probes from different sources were screened for polymorphism between the two parents (Table 1). Ninety-nine probe-enzyme combinations generated RFLP markers that segregated in the 85 mapping progeny. A total of 328 clearly scorable polymorphic loci were generated by the 99 probes. Among these markers, 232 (71%) segregated in the mapping progeny. A total of 96 bands polymorphic between the parents did not segregate in the progeny, suggesting that they are either triplex or quadruplex. Triplex markers are not expected to segregate in the progeny of tetraploids unless there was double reduction resulting from random chromatid segregation. Quadruplex markers are not expected to result in observable segregation in the offspring of tetraploid crosses. Segregating bands were separated according to their presence in either one or both parents and their segregation in the progeny. The observed segregation of markers scored in each of the parents is summarized in Fig 1. The distribution of markers in both parents exhibited a peak in the class 51–55% presence indicative of simplex markers. Fifty-three markers (22.8%) present in either one of the parents did not fit the 1:1 ratio expected for simplex markers or the 5:1 segregation ratio expected for double-dose markers at $P = 0.05$. Applying Mather's criterion for differentiating between duplex and simplex markers (see Materials and methods), 49 of these had presence to absence ratios below 2.24:1 and were retained as single-dose markers (S1 and S2). Among these 49 markers, 18 were skewed toward ratios below a 1:1 presence to absence and were marked with a star on the map. Seven fragments that were present in both parents and segregated in the progeny fit the 3:1 ratio, which would have resulted from the segregation of a SDRF in each parent (simplex-by-simplex). The number of duplex markers was very low. Only seven markers polymorphic between the two parents fit the 5:1 ratio or had a segregation ratio above 2.24:1 ($P = 0.05$).

Linkage relationship between markers

Two linkage maps were generated separately based on simplex markers from each parent. A total of 116 single dose fragments generated from 81 RFLP probes were mapped in the maternal parent AP13. A total of 109 single dose fragments generated by 64 probes were mapped in the paternal parent VS16. In the maternal parent (AP13), grouping with a LOD score of 5 and a maximum distance of 25 cM assigned 45 markers into 11 cosegregation groups. Decreasing the LOD score to 3 while keeping the maximum distance at 25 cM did not add any new linkages. In the paternal parent (VS16), grouping with the LOD score of 5 assigned 57 markers into 16 cosegregation groups. The same linkages remained when the LOD score was decreased to 3. The resulting map of AP13 consisted of 45 markers assigned to 11 linkage groups covering 412.4 cM. Seventy simplex markers remained unlinked. The size of the cosegregation groups ranged from 5.8 to 126.6 cM and the genetic distance between markers ranged from 1.3 to 33.3 cM.

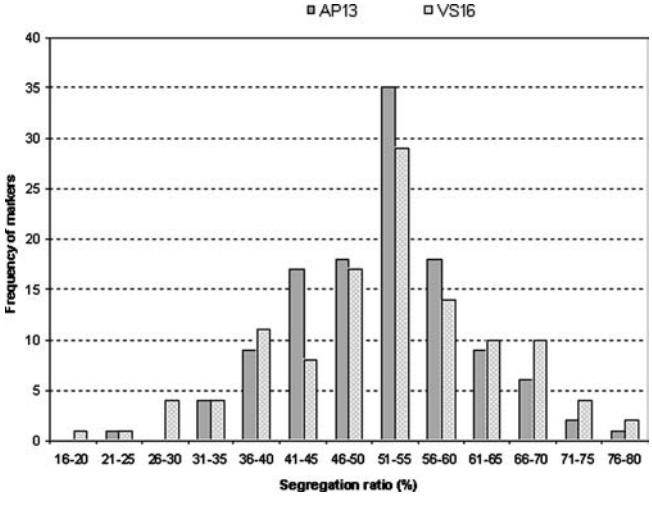


Fig. 1 Distribution of observed segregation ratios for 118 markers present in the female parent Alamo P13 and 114 markers segregating in the male parent VS16 switchgrass

Table 1 Summary of probes surveyed and mapped in the progeny of a cross between lowland Alamo (AP13) and upland Summer (VS16) switchgrass

Origin of probes	Probes tested	No signal or non-scorable bands	Non-polymorphic between parents	No segregation in the progeny	Mapped
Pennisetum cDNA (pPAP)	39	8	18	5	8
Bermuda grass (pCD)	60	45	6	2	7
Bermuda grass (T574)	67	51	3	3	10
Rice cDNA (RZ)	223	129	11	9	74
Total	389	233	38	19	99

Cosegregation groups contained from 2 to 18 markers. The VS16 map consisted of 57 simplex markers assigned to 16 linkage groups covering 466.5 cM. Fifty-one simplex markers remained unlinked. The size of the groups ranged from 2.4 to 80.3 cM, and the genetic distance between markers ranged from 1.0 to 26.7 cM. The groups contained from two to eight markers. For both maps, two markers were removed from the data because they were redundant (identified by the same probe and mapping to the same location). The cosegregation groups were named A_x for the AP13 map and S_x for the VS16 map and numbered arbitrarily according to their output in MAPMAKER (Fig. 2).

Homology between linkage groups

Assembly of homology groups is usually based on two or more common loci that are detected by the same probe and carried on different cosegregation groups. The two maps were combined based on markers identified by the same probe (Fig. 3). The parents used in this mapping study are both believed to be tetraploids ($2n=4$, $x=36$), therefore up to four homologous cosegregation groups from each parent are expected for each linkage group. Gathering cosegregation groups on the basis of common markers led to the identification of eight homology groups among the expected nine basic groups in switchgrass. The homology groups were labeled arbitrarily as linkage group + number (LG_x; Fig. 3). The largest of the homology groups (LG1) contained four cosegregation groups from AP13, two cosegregation groups from VS16, and two unlinked markers from AP13 that showed repulsion-phase linkage with groups A6 and A7. The smallest (LG3) contained only two cosegregation groups from VS16. Two cosegregation groups from AP13 (A4 and A10), one cosegregation group from VS16 (S11), and six markers from AP13 that showed repulsion-phase linkage were not assigned to any homology groups because they did not contain enough information. The order of loci among homologs sharing two or more markers was consistent and no inversions were observed. The information provided by the individual cosegregation groups did not enable the generation of a composite map for each of the different homology groups.

The assignment of cosegregation groups into homology groups based solely on common loci identified by the same probe is very sensitive and may be misleading since homologous regions may not be present only in homologous chromosomes because of the possibility of gene duplication involving non-homologous chromosomes (Pichersky 1990; Da Silva et al. 1993). The mapping of additional markers may suggest revision to the present assemblies.

Preferential pairing

The study of repulsion linkage between pairs of markers generated by the same probe or linked on homologous groups allowed the investigation of chromosome assortment and pairing behavior within homology groups. In order to determine repulsion linkage, we first

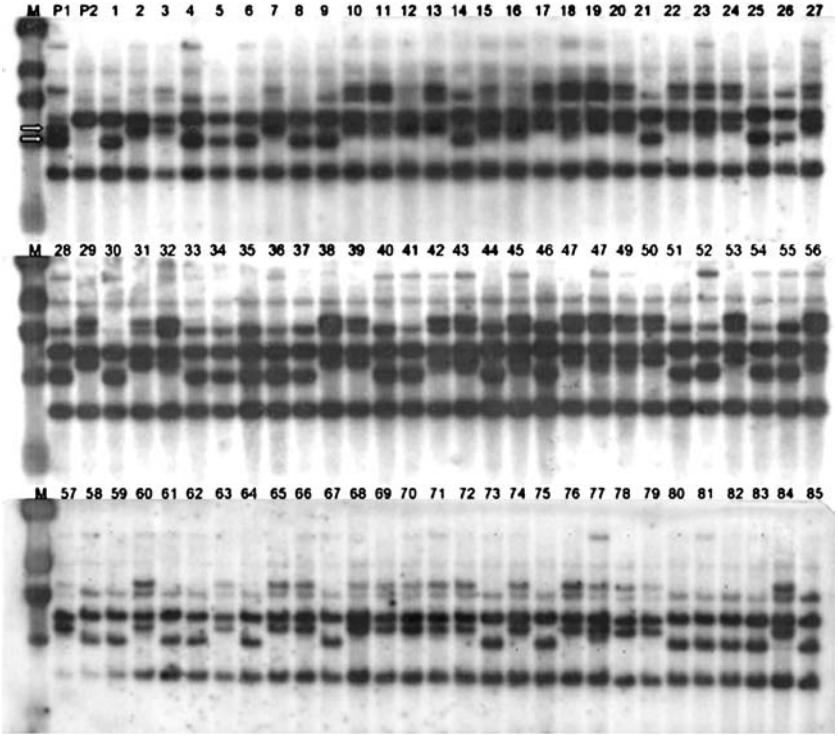


Fig. 2 Segregation of the rice probe RZ448 hybridized to genomic DNA of the parents Alamo (AP13) and Summer (VS16) and their 85 full-sib progeny digested with the restriction enzyme *Xba*I. *Lanes*: *M* lambda/*Hin*dIII ladder, *P1* female parent AP13, *P2* male parent VS16, *1–85* the 85 progeny. *Arrows on the left* point to single dose restriction fragments

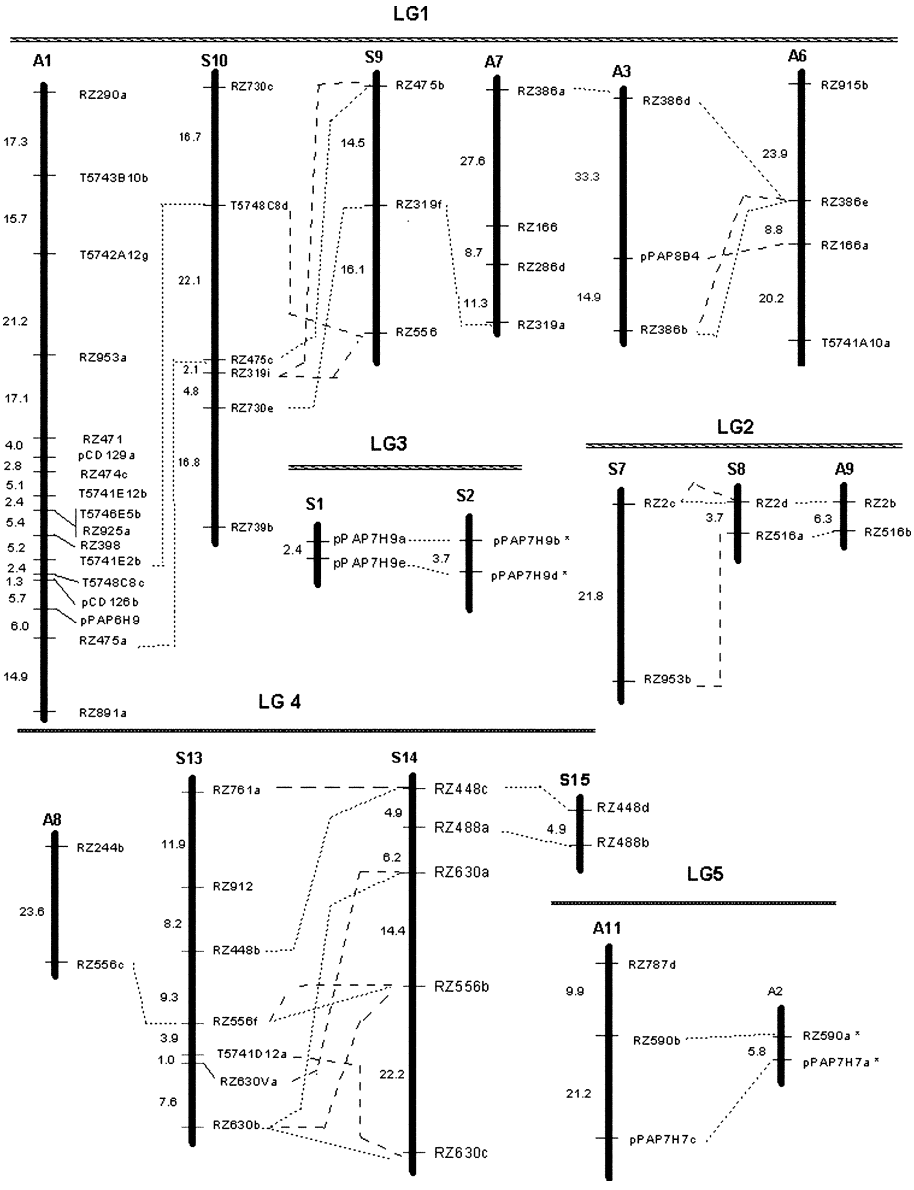
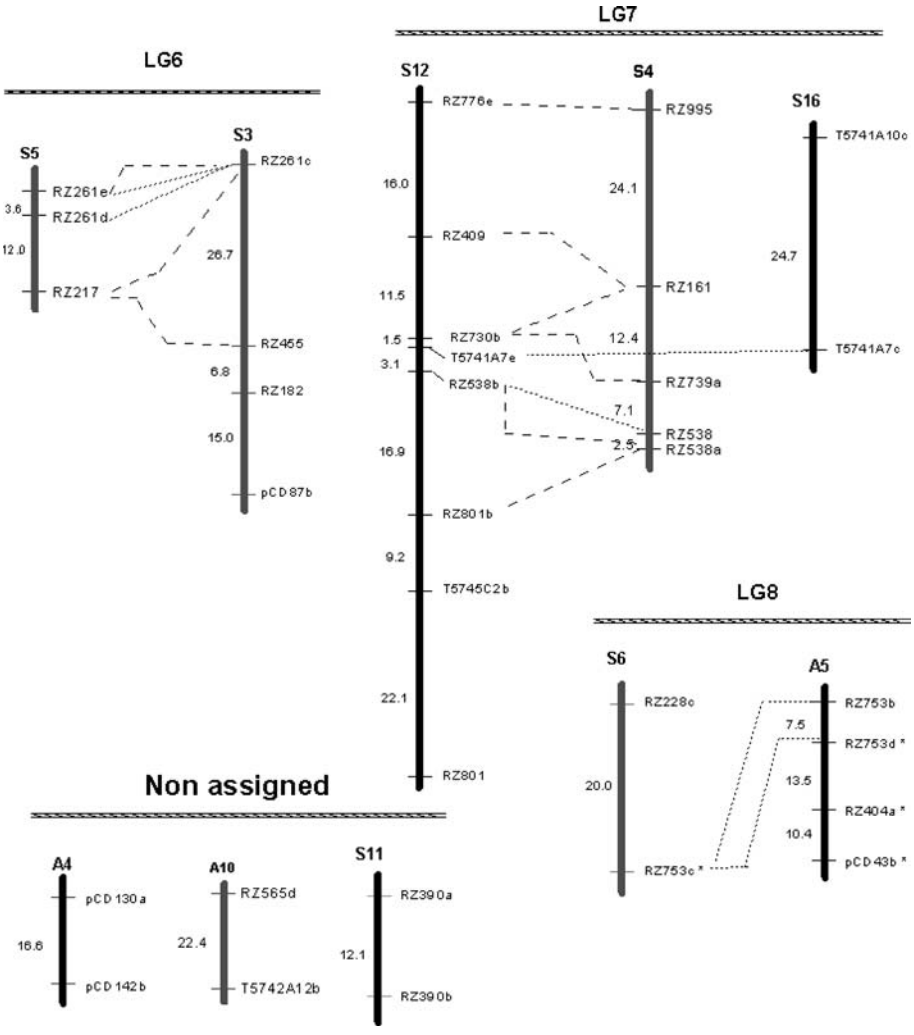


Fig. 3 Combined RFLP linkage map of Alamo AP13 and Summer VS16 switchgrass derived from 85 F_1 progenies. Cosegregation groups are denoted as A_x for Alamo and $S_x$for Summer. Groups belonging to the same linkage group are joined by a *horizontal line* and labeled LG_x. Marker names are shown on the *right* of each group. Genetic distances in centiMorgans are shown on the *left*. Markers with an *asterisk* (*) are distorted toward a lower presence to absence ($P = 0.05$). Markers with the *RZ prefix* are rice clones, with the *pPAP prefix* are *Pennisetum* clones, with the *pCD* and *T574* prefixes are Bermuda grass clones. Markers followed by a suffix (*a*, *b*) represent multiple loci detected by the same probe. *Dotted lines* connect SDRF markers detected by the same probe, *dashed lines* indicate markers that are linked in repulsion

generated a new data set by inverting the scores of the simplex markers of the original data set (Al-Janabi et al. 1994; Grivet et al. 1996; Ming et al. 1998). The two data sets were then combined and analyzed in MAPMAKER. Each pair of repulsion-phase markers were examined individually. We took into account markers involved in cosegregation groups as well as unlinked simplex markers that could be borne by undetected homologous chromosomes. A LOD threshold of 3 and a maximum recombination distance of 0.35 were used to detect the linkages. If switchgrass was an autotetraploid, setting the default linkage below 0.33 will not lead to detection of repulsion linkages even in a large population with a large number of markers because the recombination fraction due to independent assortment of repulsion markers is equal to 0.33 (Qu and Hancock 2001). Pairs of markers linked in repulsion and the statistics associated with them are listed in S3. In AP13, 17 pairs of markers were linked in repulsion. Markers on seven out of the 11 linkage groups (64%) showed evidence of strong preferential pairing with each other or with unlinked markers. Among the unlinked markers, seven pairs showed preferential pairing with one another (S3). In VS16, 25 pairs of markers linked in repulsion were detected. Twelve of the 16 cosegregation groups (75%) and two pairs of unlinked markers showed preferential pairing (S3). The repulsion linkage between pairs of markers that were assigned to cosegregation groups confirmed the assignment of these linkage groups into homology groups. Estimation of actual genetic distances and the ordering of dominant simplex markers in repulsion was not feasible. In order to place repulsion phase markers directly on a genetic map, the degree of preferential pairing in this species must be known, and the distance between markers in repulsion-phase must be expressed in terms of genetic distance rather than the

Fig. 3 (Contd.)



observed recombination fraction (Qu and Hancock 2001). A total of 96 polymorphic markers detected in the parents showed no segregation in the progeny, presumably indicating that they are polymorphisms in higher dosage than duplex and that no double reduction has occurred. This further supports data from repulsion-linkage analysis in suggesting that chromosome segregation in switchgrass involves preferential pairing. Therefore, no major map distance distortions are expected in the mapping study of this population (Yu and Pauls 1993; Lu et al. 2002).

Type of polyploidy in switchgrass

In order to distinguish between autopolyploids and allopolyploids using molecular markers, two methods have been suggested. The first is based on a comparison of the number of marker loci linked in coupling to the number of loci linked in repulsion (Wu et al. 1992). The second is based on comparing the proportion of single to multiple dose markers (Da Silva et al. 1993). In autotetraploids, multiple-dose fragments are duplex, triplex, and quadriplex. Fragments with three and four doses are expected to be found in all gametes. Only double-dose fragments are expected to be absent in one-sixth of the gametes. Therefore, the expected proportion of non-SDRF markers in the gametes of an autotetraploid is 0.17 (1/6 double dose + 0/6 triple dose). The theoretical proportion of polymorphic SDRF expected in an autotetraploid is therefore 0.83. The expected ratio of non-SDRF in an alloploid is 0.25.

In the current switchgrass mapping population, the proportion of single- to double-dose markers is significantly different from the expected ratios of both autoploid and alloploids, but there is a trend toward autoploidy (lower χ^2 values) (Table 2). The skewed ratio toward a much higher proportion of SDRF compared to double-dose markers could be influenced by omitting a number of bands that were not clearly scorable. The observed ratio of detectable SDRF linkages in coupling is expected to be equal to repulsion linkages (1:1) in allopolyploids (Wu et al. 1992). This ratio is expected to be 0.25:1 (repulsion:coupling) in autotetraploids and 0:1 in higher ploidy levels (Wu et al. 1992). In the AP13 map, the observed ratio of detectable SDRF pairs linked in repulsion and coupling was 0.16:1 and was not significantly different from the autotetraploid ratio

Table 2 Summary of Chi-squared tests of simplex to multiplex ratios, and repulsion to coupling ratios, observed in switchgrass mapping population compared to the expected ratios in autotetraploids and allotetraploids (*ns* not significant)

Criteria	Observed	Autopolyploid		Allopolyploid	
		Expected	χ^2	Expected	χ^2
Simplex to multiplex ratio					
Alamo P13					
Simplex	109	92.13		83.25	
Multiplex	2	18.87	18.16**	27.75	> 25**
	111	111		111	
Summer VS16					
Simplex	102	88.81		80.25	
Multiplex	5	18.19	11.52**	26.75	23.4**
	107	107		107	
Repulsion to coupling linkage					
Alamo P13					
Repulsion	17	25		62.5	
Coupling	108	100	3.20 ns	62.5	> 25**
	125	125		125	
Summer VS16					
Repulsion	25	26.4		67	
Coupling	107	105.6	0.09ns	67	> 25**
	132	132		132	

*Significant at $P = 0.05$, **significant at $P = 0.01$

(Table 2). In the VS16 map, the ratio of repulsion to coupling linkage was (0.23:1) and was significantly different from the expected ratio of alloploids (Table 2).

Recombinational length and marker coverage

The recombinational length of the switchgrass genome was estimated based on the paternal map of VS16 only because it has a better distribution of markers and a higher number of cosegregation groups than the maternal map. Crucial assumptions for recombination length estimation using the method-of-moment estimation is the random distribution of markers and the mutual independence between locus pairs (Charkravarti et al. 1991). Setting the LOD score to $Z = 3$, 4, and 5 gave length estimates of 4,688 cM, 4,733 cM, and 4431 cM, respectively, leading to an average of 4,617 cM.

The expected proportion of the switchgrass genome covered by the 57 single-dose markers was estimated to be 27% given an estimated recombination length of 4,617 cM, an expected 36 chromosomes in switchgrass, and a distance of 25 cM between the markers. In order to link 95% of the estimated switchgrass genome to a marker at 15 cM distance, which would be suitable for quantitative trait locus (QTL) analysis and marker-assisted applications (Beckmann and Soller 1983), a minimum of 459 markers should be placed on the map.

Linkage similarity between switchgrass and other grasses

The linkage relationship of switchgrass compared to rice, maize, and sorghum was examined using common probes that were mapped in the different species. Of the 99 probes mapped in switchgrass, 35 (35%) revealed conserved regions in other grasses (Table 3). In the combined map, *Pennisetum* clones (pPAP) that mapped to switchgrass linkage groups LG1 (A1, A3), LG3 (S1, S2), and LG5 (A2, A11) were also mapped on sorghum linkage groups A, C, G, I, J, and F. A total of 32 rice (RZ) clones that were assigned to seven linkage groups in switchgrass were also mapped on nine linkage groups in rice, nine linkage groups in maize, and five linkage groups in sorghum (Table 3). Fourteen of the rice (RZ) clones were assigned to nine cosegregation groups of the AP13, and 24 were assigned to 15 cosegregation groups of the VS16 parent. One region of 6.3 cM on switchgrass LG2 (A9) detected by the probes RZ2 and RZ516 corresponded to a region of 10.1 cM on chromosome 6 of rice. Markers RZ398 and RZ953 detected a region of 62 cM on group LG1 (A1) of switchgrass that corresponded to a region of 48.5 cM on chromosome 6 of rice. Two regions of 44.1 cM and 23.6 cM on cosegregation groups A6 and A5 that were assigned to homology groups LG1 and LG8 of switchgrass corresponded to a region of 27.3 cM on chromosome 2 and a region of 17.1 cM on chromosome 5 of rice. A total of eight regions in eight cosegregation groups of the VS16 have corresponding regions in rice chromosomes 1, 3, 5, and 6.

Discussion

We report here an early investigation into the genomic organization and chromosomal transmission in switchgrass (*Panicum virgatum* L.) based on RFLP markers. Switchgrass has not received much attention in genetic research, despite its agricultural, bioenergetic, and environmental value. As in most outcrossing polyploid species with a heterozygous genome, molecular marker analysis is complex. Uncertainty about parental linkage phases among markers is further complicated by factors

Table 3 RFLP probes mapped in switchgrass and their corresponding locations in the rice, maize, and sorghum linkage groups

Marker	Switchgrass	Rice[a,b]	Maize[b]	Sorghum[c,d]
pPAP6H9	LG1 (A1)			G
RZ398	LG1 (A1)	6		
RZ474c	LG1 (A1)	3		C
RZ953a	LG1 (A1), LG2 (S7)	6		
RZ590a	LG1 (A1), LG5 (A2)	4	2, 10	
RZ475a	LG1 (A1, S9, S10)	1		
pPAP8B4	LG1 (A3)			F
RZ386b	LG1 (A3, A6, A7)	2		
RZ166	LG1 (A6, A7)	2		F
RZ319a	LG1 (A7, S9, S10)	3		
RZ730b	LG1(S10), LG7(S120	1		
RZ2b	LG2 (A9, S7, S8)	6	5, 6, 9	
RZ516a	LG2(A9, S8)	6	9	I
pPAP7H9a	LG3 (S1, S2)			C, J
RZ244b	LG4 (A8)	5		A
RZ556c	LG4(A8)	5		
RZ761a	LG4 (S13)	3		
RZ912	LG4 (S13)	3	1, 5	
RZ630a	LG4(S13, S14)	3	1, 3, 4, 5	
RZ448b	LG4 (S13, S14, S15)	3		
RZ488a	LG4 (S14, S15)	7		
pPAP7H7c	LG5 (A2, A11)			A, G, I
RZ182	LG6 (S3)	5		
RZ261c	LG6 (S3)	12	10	
RZ455	LG6 (S3)	5	6, 8	
RZ217	LG6 (S5)	2		
RZ409	LG7 (S12	1		
RZ776e	LG7 (S12)	1		A
RZ801b	LG7 (S12)	1		
RZ161	LG7 (S4)	1		
RZ995	LG7 (S4)	1	3, 8	
RZ739a	LG7 (S4), LG1 (S10)	1		
RZ538	LG7 (S4, S120	1	3, 8	
RZ404a	LG8 (A5)	9		C
RZ753b	LG8 (A5, S6)	7	7	
RZ390a	S11	5	3, 8	

[a] Causse et al. (1994)
[b] Van Deynze et al. (1998)
[c] Ming et al. (1998)
[d] Bowers et al. (2003)

such preferential pairing between homologous chromosomes and double reduction that lead to the distortion of the segregation ratios needed to estimate recombination fractions.

Segregation distortion in switchgrass

The large number of fragments deviating from the expected ratios (23%) indicates that segregation distortion is very common in switchgrass. Segregation distortion may be due to gametophytic competition or sporophytic selection (Taylor and Ingvarsson 2003). The extent of distortion is influenced by sex and by parental interactions, as has been shown in *Pennisetum* species (Liu et al. 1996). In the present study, the number of loci showing distorted segregation is slightly higher in the male parent than the female parent (27 vs. 22). In many grasses, including *Aegilops* and wheat, preferential transmission of gametes is affected by genetic factors such as the 'cuckoo' chromosomes, which make the gametes lacking them in a heterozygous or hemizygous condition non-functional, and therefore favoring the transmission of only the gametes containing the gene (King et al. 1991).

Basic chromosome number in switchgrass

The individual linkage maps reported in this study consist of 11 and 16 cosegregation groups in AP13 and VS16, respectively. By combining the two maps based on common markers identified by the same probes, we were clearly able to identify eight homologous groups out of the nine haploid chromosome sets expected in switchgrass. Chromosome numbers have been determined for fewer than one-half of the species of the genus *Panicum*. Several studies have revealed nine to be the basic haploid number, but frequent deviations have been reported (Church 1929; Burton 1942). Zuloaga et al. (1989) studied the cytology of *Panicum validum* in order to determine its systematic position within the genus. They reported chromosome counts of $2n = 20$ with a basic number ($x = 10$). They also reported that the karyotype is symmetrical and uniform with metacentric and submetacentric chromosomes, a characteristic feature in most karyotypes of the Poaceae family. Warmke (1954) suggested $x = 8$ to be the basic chromosome number for *P. maximum* based on the study of two collections with $2n = 32$ and 48, respectively.

Several different chromosome numbers and ploidy levels have been reported for switchgrass. Nielson (1944)

noted the presence of polyploid series ranging from $2n = 18$ to $2n = 36$, 54, 72, 90, and 108. Church (1940) found somatic chromosome complements of 36 and 72 in accessions originating from Kansas and Oklahoma. Burton (1942) reported somatic counts of 72 chromosomes in a *P. virgatum* plant originating from Florida. Meiotic analysis of switchgrass collections indicated that the cytological differences and variation in chromosome numbers are associated with ecotypes. Lowland ecotypes are mainly tetraploids ($2n = 4$, $x = 36$) with a DNA content of 3 pg, whereas upland ecotypes contain octaploids ($2n = 8$, $x = 72$) and aneuploid variants of octaploids with a DNA content of 5 pg (Brunken and Estes 1975; Hopkins et al. 1996). Brown (1948) reported aneuploid somatic complements of 21, 25, 30, and 32 and noted that some of the chromosomes appeared to be fragments. Barnett and Carver (1967) also reported aneuploid complements of chromosomes of 68, 70, 76, and 78. They also observed telophase I bridges in 13 of 32 octaploid switchgrass plants, which in one plant occurred in over 35% of the cells. They did not observe bridges in any of the tetraploids, hexaploids, and aneuploids they examined.

The nature of switchgrass ploidy, auto- versus allopolyploidy, has not previously been established. In the present study, by combining information based on the simplex to multiplex ratio and repulsion to coupling linkages, we were able to infer that switchgrass is more likely to be an autotetraploid with a high degree of preferential pairing between homologous chromosomes.

Preferential pairing

In diploids and alloploids, each dominant marker has only one recessive homolog; therefore, the marker image of the dominant simplex fragment can be used to simulate the homologous recessive allele and detect repulsion-phase linkages. In autotetraploids, each dominant single-dose marker has three homologous recessive alleles, therefore using the marker image of the dominant simplex marker to simulate the homologous recessive alleles is only an approximation, and the calculation of repulsion-phase linkage may not be accurate (Krieger et al. 2000). The high incidence of repulsion linkages detected in the present study indicates that preferential pairing between homologous chromosomes of switchgrass is frequent. In reciprocal crosses between lowland Kanlow (tetraploid) and upland Summer (tetraploid) plants, Martinez-Reyna et al. (2001) found that chromosome pairing was primarily bivalent in all of the hybrids, indicating a high degree of genome similarity between upland and lowland.

In the current study, not a single double reduction event was observed, thereby providing strong support for the suggestion of preferential pairing between chromosomes. Polymorphic markers present in triple dose in one parent and absent in the other (homozygous recessive) are useful in detecting double reduction in tetraploid crosses. Double reduction is a phenomenon associated with multivalent pairing of homologous chromosomes that leads to two sister chromatids ending up together in the same gamete (Mather 1935). It leads to an increase in the frequency and distribution of homozygous gametes as compared to what is expected under random chromosome segregation.

Recombinational length of the switchgrass genome

The number of markers necessary to map a genome depends on its genome size (Bishop et al. 1983). Therefore, a preliminary estimate of the number of centi-Morgans in the genome is useful for designing linkage experiments. The estimated recombinational length of 4,617 cM for switchgrass is distributed over 36 chromosomes, giving an average chromosome size of 128 cM. The largest linkage group identified in this mapping study was 126.6 cM in the AP13 map.

Linkage similarity of switchgrass to other grasses

Comparative mapping provides an important basis for combining genetic information from different related species into consensus maps that can be useful for the cross-referencing of genetic information from distantly related species. The rice genome provides an excellent basis for comparative mapping in monocots because of its small, diploid genome (0.45 pg per haploid cell) (Arumuganathan and Earle 1991), well-characterized classical and molecular maps (Causse et al. 1994; Van Deynze et al. 1998), and nearly completed sequence.

The use of heterologous probes to generate RFLP markers in the present study showed that several genomic regions in switchgrass are composed of clones located on rice syntenic regions. Nevertheless, more anchor probes need to be placed on most of the switchgrass linkage groups in order to conduct a more comprehensive comparative analysis. Comparative maps in other members of the Panicoideae subfamily to which switchgrass belongs have been developed. These include crops such as maize, sorghum, and sugarcane (Whitkus et al. 1992; Ming et al. 1998). Much of the homology between the polyploid sugarcane and the diploid sorghum has been shown based on common probes (Ming et al. 1998).

Several studies have suggested that gene order is well-conserved within higher plant families such as the crucifers, *Arabidopsis* and *Brassica* (Kowalski et al. 1994; Lagercrantz et al. 1996; Lan and Paterson 2000) and among grasses (Keller and Feuillet 2000). Even across greater taxonomic distances, discernible similarities remain (Paterson et al. 1996; Bowers et al. 2003), indicating that the transfer of genetic information across species and genera and genomic cross-referencing between well-characterized model plants and crop species where more agronomic traits have been mapped may be possible.

The information on the genomic constitution of switchgrass presented in this study provides a framework for basic and applied genetic studies and establishes a foundation for extending genetic mapping in this crop.

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