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Application of synteny across Poaceae to determine the map location of a sugarcane rust resistance gene

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Abstract A major rust resistance gene has been identified in a self-progeny of the sugarcane cultivar R570. Until now, this gene was known to be linked to a marker revealed by the sugarcane probe CDSR29 but unassigned to any linkage group of the current genetic map. We used synteny relationships between sugarcane and three other grasses in an attempt to saturate the region around this rust resistance gene. Comparison of sugarcane, sorghum, maize and rice genetic maps led to the identification of homoeologous chromosome segments at the extremity of sorghum linkage group D, rice linkage group 2, maize linkage group 4 and in the centromeric region of maize linkage group 5. One hundred and eighty-four heterologous probes were selected and tested for cross-hybridization with sugarcane DNA; 106 produced a good hybridization signal and were hybridized on 88 individuals of the R570 selfed progeny. Two hundred and seventeen single-dose markers were added to the R570 genetic map, of which 66% mapped to linkage group VII, together with the rust resistance gene. This gene has now been mapped to the end of a co-segregating group consisting of 19 RFLP markers. None of the mapped loci were located closer to the gene than CDSR29. The gene thus appears to reside at the edge of a ''synteny cluster'' used to describe the different grass genomes.

Key words Sugarcane · Rust resistance gene · Comparative mapping · RFLP

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Introduction

Sugarcane, *Saccharum* spp., is a highly polyploid and aneuploid grass of the *Andropogoneae* tribe. Modern cultivars are interspecific hybrids involving essentially a domesticated species, *S. officinarum* L. (2n = 80) (high sugar content), and a wild species, *S. spontaneum L.* (2n = 40 to 128) (resistant to biotic and abiotic stresses), each with different basic chromosome numbers (10 and 8, respectively; D'Hont et al. 1998). The chromosome number of these interspecific hybrids ranges from 2n = 100 to 2n = 130; the proportion of chromosomes derived from *S. spontaneum* is minor (around 15%) compared to that from *S. officinarum* (D'Hont et al. 1996). Despite the structural complexity of the sugarcane genome, genetic maps of three *Saccharum* species, *S. officinarum* (Guimaraes et al. 1997; Ming et al. 1998), *S. spontaneum* (Da Silva et al. 1993, 1995; Al-Janabi et al. 1993; Ming et al. 1998) and *S. robustum* Brandes and Jeswier (Guimaraes et al. 1997; Ming et al. 1998) and one modern cultivar, R570 (Grivet et al. 1996), have been built with restriction fragment length polymorphism (RFLP) and/or random amplified polymorphic DNA (RAPD) markers. R570 is the most utilized sugarcane cultivar in Reunion Island and Mauritius and is included as a progenitor in many breeding programs.

The molecular genetic map of R570 was constructed with a set of 128 maize and sugarcane RFLP probes. Four hundred and eight simplex markers were mapped on 96 co-segregation groups assembled into ten linkage groups on the basis of common probes (Grivet et al. 1996). A recent addition of 460 markers (322 RFLP and 138 AFLP markers) reduced the number of apparent linkage groups to nine (unpublished data).

A major rust resistance gene was identified on the self-progeny used to construct the genetic map of the cultivar R570 (Daugrois et al. 1996); it is the first major resistance gene to be described in sugarcane. Leaf rust in sugarcane is caused by the pathogenic agent *Puccinia melanocephala* H. & P. syd. and is characterized by sporulant rust-colored pustules on the leaf surface of susceptible individuals. The segregation ratio of resistance in the progeny was three resistant to one susceptible, indicating that the allele conferring resistance to this disease is dominant and that there is one sole copy in the genome of R570. This resistance gene was lo-

cated on the R570 genetic map; it was linked to a marker revealed by the sugarcane probe CDSR29, itself left unassigned to any defined linkage group.

Colinearity and synteny between related species have been largely studied through comparative mapping, specifically within the *Poaceae* family that contains many of the more important cereal crops. With respect to the *Andropogoneae* tribe, early reports focused on sorghum and maize (Hulbert et al. 1990; Binelli et al. 1992; Whitkus et al. 1992; Melake-Berhan et al. 1993; Pereira et al. 1994) and demonstrated that the two species share many orthologous loci arranged in colinear regions forming, however, a rather complex patchwork. D'Hont et al. (1994) compared the sugarcane and maize genomes and were the first to demonstrate that sugarcane genome analysis could benefit from comparative analyses with simpler species. Comparative genome mapping between sugarcane and sorghum (Dufour et al. 1997; Guimaraes et al. 1997; Ming et al. 1998) has demonstrated a high degree of synteny and colinearity. Several comparative studies have been were performed between sugarcane, sorghum and maize (Grivet et al. 1994; Dufour et al. 1996; Glaszmann et al. 1997), and these have shown that sugarcane and sorghum are more closely related to each other than either one is to maize. Comparative genome mapping has also been carried out between maize and rice, which belong to different tribes; Ahn and Tanksley (1993) showed that linkage conservation applies to more than two-thirds of both genomes.

The degree of genome conservation observed between these species provides the possibility of transferring genetic information and mapping resources (RFLP probes) from well-characterized genomes, such as that of maize, and/or from simple genome structure species, such as those of rice or sorghum, to less-studied and more complex genomes such as that of sugarcane, in order to saturate the genetic map or regions surrounding genes of particular interest.

The goal of the study reported here was to identify closely linked markers flanking the rust resistance gene in sugarcane to eventually initiate a chromosome walk toward the gene using the sugarcane BAC library developed on R570 (Tomkins et al. 1999). For this purpose, we exploited the synteny between sugarcane and three other grasses – sorghum, maize and rice.

Materials and methods

Plant material

The mapping population of sugarcane used in this study has been previously described by Grivet et al. (1994). It consists of 88 clones derived from a self fertilization of the rust resistant cultivar R570. This cultivar was developed by crossing H32–8560 and R445, both of which are resistant to rust.

The sorghum mapping population used to map the sugarcane probe CDSR29 is described in Dufour et al. (1997). This population, RIL379, is composed of 110 recombinant inbred lines (RIL) derived from the cross IS2807 × 379.

Probes

Among the 87 maize probes used, 11 (AGR) were from Mycogen Plant Science, 5 (ASG) from Asgrow seeds, 11 (BNL) were the from Brookhaven National Laboratory, 15 (CSU) were from California State University-Hayward, 8 (ISU) were from Iowa State University, 13 (NPI) were from Native Plants Inc. & Pioneer Hi-Bred International, 5 (PHP) were from Pioneer Hi-Bred International, 9 (UAZ) were from the University of Arizona, 6 (UMC) were from the University Missouri-Columbia and 4 genes – *BT1* from the University of Wisconsin-Madison, *Xet1* from the University of Illinois, and *dba1* and *eif*5 from the University of Missouri-Columbia, 42 and 45 probes were gDNA and cDNA clones, respectively. Among the 75 rice probes, 26 RGC, 26 RGR and 5 RGG probes were provided by the Japanese Rice Genome Mapping Project and 12 RZ and 6 RG by Cornell University; 11 and 64 probes were gDNA and cDNA clones, respectively. Among the nine sorghum probes, six (TXS) were provided by Texas A & M University and three (SbRPG) by Rustica Prograin Genetique and Cirad; Six and three probes were gDNA and cDNA clones, respectively. The 11 oat and two barley probes, CDO and BCD probes, respectively, were provided by Cornell university; these probes were cDNA clones. DNA inserts were cloned in DH5-a and amplified by the polymerase chain reaction (PCR).

Southern analysis

Genomic DNA extraction and Southern blot hybridization have been described in Hoisington et al. (1992) and Grivet et al. (1994); the wash conditions (0.5 × SSC, 65°C) were identical for all probes (Da Silva et al. 1993). Survey filters consisted of three lanes, one of maize DNA (cultivar Goldcrest) digested with *Hind*III (6 µg/lane), one of rice DNA (cv. KU86) digested with *Hind*III (3 µg/lane) and three of R570 (sugarcane cv.) DNA each digested with *Hind*III, *Dra*I or *Sst*I (10 µg/lane). These were used to screen the heterologous probes for cross-hybridization quality with sugarcane DNA and number of markers revealed. R570 progeny DNA was digested with either *Hind*III, *Dra*I or *Sst*I (10 µg/lane). Southern hybridization of sorghum DNA was performed as described in Dufour et al. (1996).

Data analysis and mapping of markers

The segregation of each scorable band was treated independently based on its presence or absence in the progeny. The genetic map was constructed with single-dose restriction fragments (SDRFs) only. A SDRF is a fragment that is present in a single copy in the parent and which segregates in a single-dose ratio, that is 3:1 (present : absent) in the selfed progeny. SDRFs were selected as in Grivet et al. (1996) and added to the segregation data of markers used to construct the current linkage map of R570. Markers produced by the same probes and displaying the same segregation pattern were considered to be redundant, and only one was conserved for analysis. Biased markers showing segregation distortion toward lower values on the basis of a χ^2 test at $P = 0.05$ (Grivet et al. 1996) were integrated in the analysis and indicated on the figures.

Linkage analysis of the markers was performed with MAPMAKER 3.0 (Lander et al. 1987). Markers were assembled into co-segregation groups by two-point analysis at a LOD score of 5. New co-segregation groups were assigned to pre-existing linkage groups on the basis of at least 2 common linked probes. Co-segregation groups assigned to a linkage group were ordered with multipoint analysis; when the most likely hypothesis was less than ten times more likely than the second one, the order was considered to be ambiguous.

Seventy-seven individuals were part of the population evaluated for resistance to rust (Daugrois et al. 1996). The other 11 individuals were taken from a larger population consisting of 700 individuals that were tested for 2 consecutive years in the field. Field trials were located at the Cirad station of La Mare and Le Gol (Réunion Island) using natural infection. Rust resistance level was evaluated using a graduated scale from 1 (the most resistant) to 9 (the most susceptible) (Tai et al. 1981).

Mapping of markers on the sorghum population was described in Dufour et al. (1996).

Comparative mapping

Comparison of sugarcane, sorghum, maize and rice was focused on the chromosome blocks homoeologous to the region of the rust resistance gene in sugarcane. A synthetic map of sorghum, maize

and rice chromosome segments involved in the comparison was constructed based on different published genetic maps of each species [sorghum: Binelli et al. 1992; Whitkus et al. 1992; Melake-Berhan et al. 1993; Xu et al. 1994; Pereira et al. 1994; Lin et al. 1995; Dufour et al. 1997; Boivin et al. 1999; maize: Hoisington and Coe 1990; Beavis et al. 1991; Burr and Burr 1991; Ahn and Tanksley 1993; Heredia et al. 1994; Chao et al. 1994, Maize Genetics Cooperation Newsletter 1996; BNL 96, Mycogen and UMC 98 maps in maize DB (http://www.agron.missouri.edu); rice: Causse et al. 1994; Kurata et al. 1994]. The initial framework of each chromosome segment was founded on the most saturated published genetic map of each species, and additional probes from others maps were added approximately.

On the basis of our own experiments, a composite map of sugarcane linkage group VII was built based on the 10 largest co-segregation groups out of the 17. The analysis was performed using the matrix [probes × cosegregation groups] as described in D'Hont et al. (1994). An initial set of 8 markers, well distributed along the linkage group, were chosen and ordered with a multipoint analysis at a threshold LOD score of 1. Other markers were added to this group and ordered with the same analysis threshold. Markers that could not be ordered at this LOD score were placed at the most likely position according to multipoint analysis and were highlighted on the map. The probes mapped on the other seven cosegregation groups and not integrated in the above procedure were added on the composite map on the basis of their relative position on the individual co-segregation groups.

Results

Probe CDSR29, which was not linked on the initial R570 genetic map, hybridized with sorghum DNA but not with maize and rice DNA. This probe was analyzed on the progeny of the sorghum cross IS2807 × 379 used to build our sorghum genetic map. It revealed 1 marker mapped at the extremity of sorghum linkage group D using the nomenclature derived from that of Pereira et al. (1994). The established homoeology relationships enabled us to target maize and rice chromosome segments homoeologous to the sorghum segment bearing the locus CDSR29: two segments on the maize map, one at the extremity of linkage group 4 and the other one in the centromeric region of linkage group 5; one segment on the rice map at the extremity of linkage group 2.

One hundred and eighty-four probes were selected in these targeted homoeologous regions: 32 on the basis of various sorghum maps, 86 on maize maps and 66 on rice maps. They include nine sorghum probes, 87 maize probes, 75 rice probes and 13 oat and barley probes (11 and two, respectively). cDNA clones (68%) were preferentially chosen because the transcribed sequences are more likely to be conserved between species than other genomic sequences.

Of the 184 probes 106 produced a good hybridization signal with sugarcane DNA and were further hybridized on our mapping population. Sixty of these produced a poor or unscorable signal for mapping, with bands too weak or background levels too high, and 18 other probes did not produce any hybridization signal at all on sugarcane DNA. The percentage of heterologous probes that cross-hybridized with sugarcane DNA (at the stringency levels described above) was 78% (7/9) for sorghum, 68% (59/87) for maize, 45% (34/75) for rice and 36% (4/11) for oat.

A total of 106 probes, involved in 115 probe/enzyme combinations, were surveyed on the R570 progeny. They produced 1,020 bands, from which 524 were polymorphic and 311 segregated 3:1. Among the latter simplex markers, 32 were redundant. On average, each polymorphic probe generated 5.5 segregating bands, of which 3.3 were simplex markers, whereas 11 probes did not reveal any polymorphic marker.

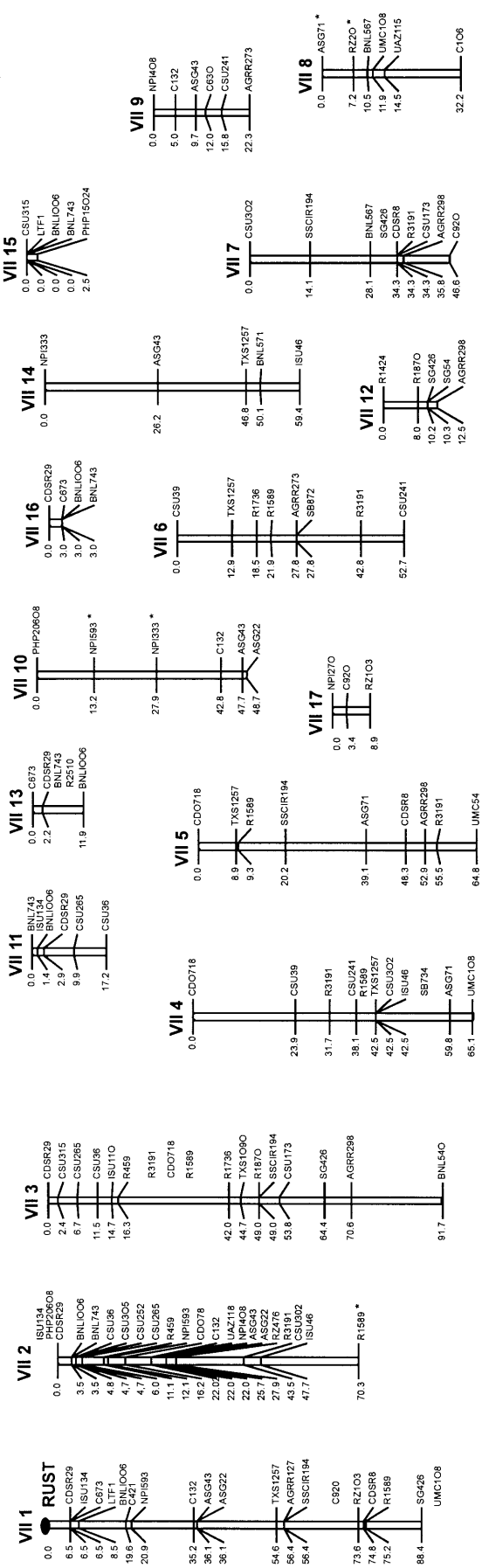
Segregation of 279 non-redundant markers with complete or near-complete data sets were analyzed using the MAPMAKER 3.0 computer program together with the 730 pre-existing RFLP markers of our current R570 map to yield as many as 508,536 pairwise comparisons.

A total of 217 markers (representing 75% of the probes analyzed) were located on co-segregation groups belonging to one of the nine linkage groups of the current R570 map, whereas 32 were placed in co-segrega-

Table 1 Distribution of the 279 markers mapped on R570 linkage groups and locus coincidence with sorghum, maize and rice homoeologous segments

Linkage group[a]	Number of markers/linkage group	Number of probes involved	Locus coincidence with homoeologous regions			
			Sorghum D	Maize 4	Maize 5	Rice 2
I	11	5	2	2	2	–
II	12	6	2	–	4	–
IV	4	2	–	1	–	1
V	4	4	1	–	3	–
VII	122	53	17	11	14	14
VIII	10	7	1	3	1	2
IX	36	18	4	3	13	–
X	13	8	2	2	4	1
XI	5	2	–	1	1	–
Total	**217**		**29**	**23**	**42**	**18**
L	32	9				
U	30	34				
Total	**279**					

[a] Roman numbers indicate sugarcane linkage groups (in accordance with Grivet et al. 1996); L indicates co-segregation groups yet unassigned to any defined linkage group; U indicates markers yet unlinked. The nomenclature of sorghum linkage groups is that of Pereira et al. (1994)



tion groups that were not yet integrated into any linkage group, and 30 markers were left unlinked (Table 1). Of the 217 markers integrated into a linkage group, 122 (representing 66% of the mapped probes) were assigned to linkage group VII and 36 to linkage group IX, while the other 59 markers were scattered among seven linkage groups.

The resulting new state of linkage group VII is as follows: it is composed of 17 co-segregation groups and encompasses 146 markers revealed by 61 probes (Fig. 1). The co-segregation group that contains the rust resistance gene consists of 19 RFLP markers (Fig. 1) covering 88 cM. Only one end of the resistance gene has been marked, and the gene itself appears to occupy the terminal position of the group. The closest marker to the gene is at 6.5 cM and is revealed by the sugarcane probe CDSR 29. There are 2 other markers at the same position, revealed by rice and maize probes C673 and ISU134, respectively. The other 16 co-segregation groups of linkage group VII vary in length from 2.5 to 92 cM and carry between 3 and 22 markers. The new markers incorporated into the co-segregation groups of linkage groups II, IV, V, VIII, IX, X and XI were also ordered. In general, they were found to be scattered on several cosegregation groups and dispersed along them (data not shown).

A composite linkage group VII map, bearing the rust resistance gene, was built and compared with the homoeologous segments of sorghum, maize and rice maps (Fig. 1). The construction of the composite linkage group was performed as described in D'Hont et al. (1994) by pooling the segregation data of the 10 largest cosegregation groups, thereby representing 55 of the 61 RFLP probes mapped on this linkage group. The other 6 probes (BNL5.71, NPI270, PHP15024, R1424, R2510 and SG54) were added in a second round on the basis of their relative position in individual cosegregation groups.

Among the 32 probes selected from the targeted area of sorghum map, 22 cross-hybridized with sugarcane DNA, and 17 revealed markers located on linkage group VII, including 5 which revealed duplicated loci (CSU36 revealed markers on linkage groups II and VII, ISU110 on I, VII and IX, CDO78 on VII and IX, BNL5.71 on II, VII and X and BNL5.67 on VII and IX) (Fig. 2). Three probes revealed markers mapped on other linkage groups, and 2 revealed markers unlinked on the R570 molecular map (Fig. 2). Colinearity of the 17 loci syntenic in both sugarcane and sorghum was very well conserved, except for 4 loci (BNL5.71, SbRPG734, SbRPG872 and ISU46) whose order was uncertain on the linkage group VII composite map. The rust resistance gene is located at the extremity of the composite linkage group VII, which also corresponds to the extremity of sorghum composite linkage group D. The terminal

Fig. 1 Cosegregation groups of sugarcane linkage group VII. Distances between markers were calculated with the Haldane mapping function. Markers whose position is not indicated with a *horizontal line* were ordered at a LOD score <1. An asterisk (*) indicates those markers showing segregation distortion and *RUST* indicates the position of the rust resistance locus

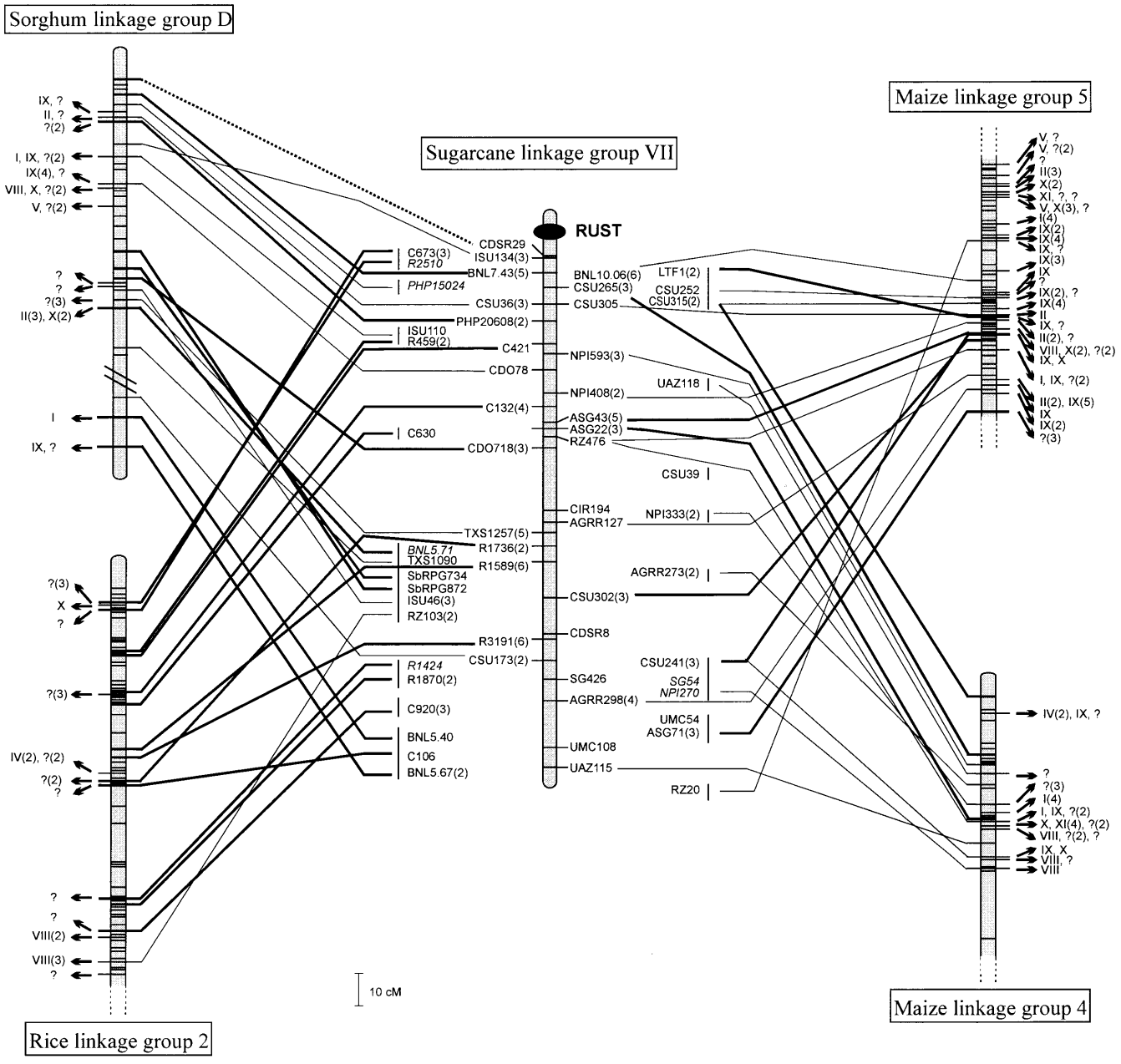


Fig. 2 Location, on the R570 sugarcane map, of the probes selected from homoeologous chromosomes in several grass species. Vertical bars represent chromosomal segments of sorghum, maize and rice relative to the sugarcane segment that bears the rust resistance locus. The maps for sorghum, maize and rice result from a synthesis of published maps. Positions of the probes are indicated with *horizontal lines – short* ones for probes that do not cross-hybridize with sugarcane DNA, and *long* ones for probes that reveal markers on sugarcane linkage group VII and/or on other linkage group (indicated to the *side* of an *arrow*). The sugarcane map uncompasses probes with specific locations and probes whose location is approximate (*vertical bars*). The *horizontal/diagonal lines* between chromosome segments indicate the position of syntenic loci between sugarcane and the other three grasses. *Thick dark lines* connect probes that have been precisely localized on homoeologous segments, and *fine lines* connect those with a relative imprecise position

probe, CDSR29, of sorghum linkage group D reveals the closest marker to the rust resistance gene in sugarcane at a distance of 6.5 cM.

Of the 86 probes selected from the targeted area of maize genetic maps, 56 could be positioned on the sugarcane R570 map; 17 belong to maize linkage group 4, 36 to linkage group 5 and 3 probes reveal a locus mapped on both linkage groups. Of the 20 probes selected from maize linkage group 4, 16 revealed markers segregating in a 3:1 ratio. Eleven of these revealed markers mapped on linkage VII, among which 3 (RZ476, CSU241 and NPI 270) revealed duplicated loci mapped on other linkage groups as well; the other 5 probes were mapped on different linkage groups (Fig. 2). Among the 39 probes selected from maize linkage group 5, 33 displayed at least 1 marker segregating in a 3:1 ratio. Fourteen of

them mapped on linkage group VII, including 6 which revealed duplicate loci into other linkage groups. Seventeen probes mapped only on other linkage groups, with a majority on linkage group IX, and 2 revealed markers unlinked on the current R570 molecular map (Fig. 2). Probes selected from maize linkage group 4 and 5 enabled us to identify 23 and 42 loci, respectively, on the R570 genetic map (Table 1). A comparison of composite sugarcane linkage group VII and linkage groups 4 and 5 of maize reveals some rearrangements between genomes of both species. The comparison with maize linkage group 4 involves 11 probes of which 5 (ASG22, RZ476, NPI333, AGRR273 and UAZ115) apparently do not respect map colinearity. However, the position of 4 of these 5 probes (RZ476, NPI333, AGRR273 and UAZ115) was uncertain on the genetic map of at least one of the two species compared. The terminal probe CSU315 is the probe that reveals the marker closest to the rust resistance gene in sugarcane among those selected from maize linkage group 4. Among the 14 common probes mapped on maize linkage group 5, 4 (LTF1, CSU302, CSU241 and RZ20) markedly reveal colinearity breaks (Fig. 2). Some of them have approximate locations, and the global pattern does not suggest any particular simple rearrangement. The marker closest to the rust resistance gene is revealed by BNL10.06. This locus is penultimate on the studied region of maize linkage group 5. The adjacent marker located just above, namely RZ20, does not respect colinearity and maps on the opposite end of the sugarcane composite linkage group VII.

Among the 66 probes selected from the rice genetic map, 28 cross-hybridized with sugarcane DNA and 19 of them revealed markers segregating in a 3:1 ratio in sugarcane. Fourteen were mapped on linkage group VII, 1 (RZ103) of which revealed another locus on linkage group VIII (Fig. 2). Three probes were mapped on linkage groups IV, VIII and X, and 2 revealed markers unlinked on R570 molecular map (Fig. 2). The order of the 14 markers mapped on sugarcane linkage group VII was conserved in rice except for 2 markers revealed by R1736 and C106. Two other probes, namely C673 and R2510, apparently do not respect map colinearity (Fig. 2), but they revealed very close markers whose location was not accurate on the sugarcane map. The rust resistance gene is localized at the extremity of the composite linkage group VII that corresponds also to the extremity of rice linkage group 2. The 2 terminal probes of rice linkage group 2, namely C673 and R2510, reveal the markers closest to the rust resistance locus.

Discussion

Our current knowledge of sugarcane, sorghum, maize and rice genetic maps enabled us to identify chromosome segments corresponding to the region that harbors the rust resistance gene in sugarcane. These segments are located at the extremity of sorghum linkage group D, rice linkage group 2, maize linkage group 4 and in the centromeric region of maize linkage group 5. The existence of two different homoeologous linkage groups of maize is consistent with the duplicated origin of this genome (Gottlieb 1982; Helentjaris et al. 1988). The use of heterologous probes selected from these regions enabled the rust resistance gene to be located at the extremity of a co-segregation group belonging to linkage group VII of the current R570 genetic map. However, none of the mapped loci was located closer to the target gene than the marker revealed by CDSR29, and only one end of the gene was marked. All of the synteny and colinearity relationships with sorghum, rice and maize linkage group 4 indicate that the resistance gene is in a subtelomeric position. The homoeologous segment identified in the centromeric region of maize linkage group 5 is adjacent to a breakpoint of synteny conservation; all probes above this breakpoint that could be mapped on R570 fell into linkage groups other than VII (Fig. 2), whereas all probes derived from below this breakpoint were mapped on a single side of CDSR29.

The efficiency of the comparative approach was slightly different between the three species used. The degree of cross-hybridization of the heterologous probes was 78%, 68% and 45% for sorghum, maize and rice, respectively (Table 1). These values are slightly lower but close to values reported in earlier studies: 78–97% cross-hybridization was observed between maize probes and sugarcane DNA (D'Hont et al. 1994; Da Silva et al. 1993; Grivet et al. 1996) with stringency conditions of 0.1 × SSC to 0.5 × SSC and 52% between rice probes and sugarcane DNA (Da Silva et al. 1993) with 0.5 × SSC. This pattern of cross-hybridization with sugarcane DNA places sorghum closer to sugarcane than maize, while rice is more distant. These results agree with the current phylogenic hypothesis. The three genera *Saccharum*, *Sorghum* and *Zea* are members of the *Andropogoneae* tribe while the genus *Oryza* belongs to the *Oryzeae* tribe.

Among the 81 heterologous probes linked on the R570 genetic map, 53 (or 65%) were assigned to R570 linkage group VII (Table 1) together with the rust resistance gene. Locus coincidence between the homoeologous regions compared was high for sorghum and rice – 60% and 78%, respectively. The same parameter amounted to 48% and 33% for maize linkage groups 4 and 5, respectively. The synteny was thus relatively well conserved between sugarcane (R570), sorghum, rice and maize linkage group 4, whereas maize linkage group 5 seems to depart. Non-syntenic probes were usually scattered on various linkage groups (Table 1) and were not clustered in particular regions of the sugarcane map. Therefore, disruption of synteny could not be explained by simple rearrangements. However, it is noteworthy that the segment of maize linkage group 5 was the source of many probes that mapped on both sugarcane linkage groups VII and IX. This may be indicative of specific processes that have affected the evolution of this region in maize.

Colinearity of syntenic loci was relatively well conserved between the different species compared (Fig. 2).

Few intrachromosomal rearrangements were observed, and some of these rearrangements could be due to the uncertain location of markers on sugarcane composite map. The global picture that we observed follows the standard described by Bennetzen and Freeling (1997), who stated that 20–40% of the DNA markers in one grass species will not be colinear with DNA markers of another grass.

Despite the failure to find markers on both sides of the target locus, the general level of colinearity is promising for developing heterologous molecular resources to assist gene analysis in sugarcane. Sugarcane cultivars have a very complex genome with around 12 homologous or homoeologous versions of each basic chromosome. Map-based cloning could be facilitated by the use of species with smaller and simpler genomes. Among the three species we used, the genetic information and molecular resources derived from sorghum appeared to be most valuable. However, the current genetic maps of sorghum are not completely saturated yet. Rice also showed a high degree of synteny and colinearity at the genetic map level with sugarcane for the region studied. Rice has a small genome (0.46 pg per basic genome compared to 0.8 pg for sorghum, 0.9 pg for sugarcane and 2.9 pg for maize; Bennett and Leitch 1995, unpublished data for sugarcane) and highly saturated physical maps (Zhang and Wing 1997). It thus appears to be the species that is at present the best adapted to be readily used to accelerate chromosome walking toward the rust resistance identified in R570.

Map-based cloning assisted by synteny also requires conservation of microcolinearity across the two species involved in the chromosome walk. Several studies have demonstrated that there can be local conservation of colinearity at the microlevel between different grass species (Kilian et al. 1995; Dunford et al. 1995; Foote et al. 1997; Gallego et al. 1998). The subtelomeric situation of the gene could, however, be a source of difficulty, since an increasing rate of recombination in distal parts of grass chromosomes, which can lead to rearrangements, has been observed (Kilian et al. 1995; Van Deynze et al. 1995; Gallego et al. 1998). When markers close enough to the resistance gene are detected in R570, it will be important to determine if synteny and colinearity are conserved between rice and sugarcane at the microlevel.

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References

Ahn S, Tanksley SD (1993) Comparative linkage maps of the rice and maize genomes. Proc Natl Acad Sci USA 90: 7980–7984

Al-Janabi SM, Honeycutt RJ, McClelland M, Sobral WS (1993) A genetic linkage map of *Saccharum spontaneum* L. 'SES 208'. Genetics 34: 1249–1260

Beavis WD, Grant D, Albertsen M, Fincher R (1991) Quantitative trait loci for plant height in four maize populations and their associations with qualitative genetic loci. Theor Appl Genet 3: 141–145

Bennett MD, Leitch IJ (1995) Nuclear DNA amount in angiosperms. Ann Bot 76: 113–176

Bennetzen JL, Freeling M (1997) The unified grass genome: synergy in synteny. Genome Res 7: 301–306

Binelli G, Gianfranceschi L, Pè ME, Taramino G, Busso C, Stenhouse J, Ottaviano E (1992) Similarity of maize and sorghum genomes as revealed by maize RFLP probes. Theor Appl Genet 84: 10–16

Boivin K, Deu M, Rami J-F, Trouche G, Hamon P (1999) Towards a saturated sorghum map using RFLP and AFLP markers. Theor Appt Genet 98:320–328

Burr B, Burr FA (1991) Recombinant inbreds for molecular mapping in maize. Trends Genet 7: 55–60

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138 1251–1274

Chao S, Baysdorfer C, Heredia-Diaz O, Musket T, Xu G, Coe EH (1994) RFLP mapping of partially sequenced leaf cDNA clones in maize. Theor Appl Genet 88: 717–721

D'Hont A, Lu YH, Gonzalez de Leon D, Grivet L, Feldmann P, Lanaud C, Glaszmann JC (1994) A molecular approach to unraveling the genetics of sugarcane, a complex polyploid of the Andropogoneae tribe. Genome 37: 222–230

D'Hont A, Grivet L, Feldmann P, Rao S, Berding N, Glaszmann JC (1996) Characterization of the double genome structure of modern sugarcane cultivars (*Saccharum* spp.) by molecular cytogenetics. Mol Gen Genet 250: 405–413

D'Hont A, Ison D, Alix K, Roux C, Glaszmann JC (1998) Determination of basic chromosome numbers in the genus *Saccharum* by physical mapping of ribosomal RNA genes. Genome 41: 221–225

Da Silva JAG, Sorelles ME, Burnquist WL, Tanksley SD (1993) RFLP linkage map and genome analysis of *Saccharum spontaneum*. Genome 36: 782–791

Da Silva J, Honeycutt R, Burnquist W, Al-Janabi SM, Sorells ME, Tanksley SD, Sobral WS (1995) *Saccharum spontaneum* L. ''SES 208'' genetic linkage map combining RFLP- and PCR-based markers. Mol Breed 1: 165–179

Daugrois JH, Grivet L, Roques D, Hoarau JY, Lombard H, Glaszmann JC, D'Hont A (1996) A putative major gene for rust resistance linked with a RFLP marker in sugarcane cultivar 'R570'. Theor Appl Genet 92: 1059–1064

Dufour P, Grivet L, D'Hont A, Deu M, Trouche G, Glaszmann JC, Hamon P (1996) Comparative genetic mapping between duplicated segments on maize chromosomes 3 and 8 and homoeologous regions in sorghum and sugarcane. Theor Appl Genet 92: 1024–1030

Dufour P, Deu M, Grivet L, D'Hont A, Paulet F, Bouet A, Lanaud C, Glaszmann JC, Hamon P (1997) Construction of a composite sorghum genome map and comparison with sugarcane, a related complex polyploid. Theor Appl Genet 94: 409–418

Dunford RP, Kurata N, Laurie DA, Money TA, Minobe Y, Moore G (1995) Conservation of fine-scale DNA marker order in the genomes of rice and Triticeae. Nucleic Acids Res 23: 2724–2728

Foote T, Roberts M, Kurata N, Sasaki T, Moore G (1997) Detailed comparative mapping of cereal chromosome regions corresponding to the *Ph1* locus in wheat. Genetics 147: 801–807

Gallego F, Feuillet C, Messner M, Penger A, Graner A, Yano M, Sasaki T, Keller B (1998) Comparative mapping of the two wheat leaf rust resistance loci *Lr1* and *Lr10* in rice and barley. Genome 41: 328–336

Glaszmann JC, Dufour P, Grivet L, D'Hont A, Deu M, Paulet F, Hamon P (1997) Comparative genome analysis between several tropical grasses. Euphytica 96: 13–21

Gottlieb LD (1982) Conservation and duplication of isozymes in plants. Science 216 : 373–380

Grivet L, D'Hont A, Dufour P, Hamon P, Roques D, Glaszmann JC (1994) Comparative genome mapping of sugarcane with other species within the Andropogoneae Tribe. Heredity 73:500–508

Grivet L, D'Hont A, Roques D, Feldmann P, Lanaud C, Glaszmann JC (1996) RFLP mapping in cultivated sugarcane (*Saccharum* spp.): genome organization in a highly polyploid and aneuploid interspecific hybrid. Genetics 142: 987–1000

Guimaraes CT, Sills GR, Sobral BW (1997) Comparative mapping of Andropogoneae: *Saccharum* L. (sugarcane) and its relation to sorghum and maize. Proc Natl Acad Sci USA 94: 14261–14266

Helentjaris T, Weber D, Wright D (1988) Identification of the genomic locations of duplicate nucleotide sequences in maize by analysis of restriction fragment length polymorphism. Genetics 118: 353–363

Heredia DO, Gardiner J, Hoisington D, Chao SM, Coe E, Musket T, Xu GL (1994) Combined f2 and if2 RFLP map. Maize Genet Coop Newsl 68: 30—35

Hoisington D (1992) Laboratory protocols. CIMMYT Appllied Molecular Genetics Laboratory, CIMMYT, Mexico DF

Hoisington DA, Coe EH (1990) Mapping in maize using RFLP's. In: Gustafson JP (eds) Gene manipulation in plant improvement. Plenium Press, New York, pp 331–351

Hulbert SC, Richter TE, Axtell JD, Bennetzen JL (1990) Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. Genetics 87: 4251–4255

Kilian A, Kudrna DA, Kleinhofs A, Yano M, Kurata N, Steffenson B, Sasaki T (1995) Rice-barley synteny and its application to saturation mapping of the barley *Rpg1* region. Nucleic Acids Res 23: 2729–2733

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin SY, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang ZX, Monna T, Umehara Y, Yano M, Sasaki T, Minobe Y (1994) A 300-kilobase interval genetic map of rice including 883 expressed sequences. Nat Genet 8: 365–372

Lander E, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newberg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174–181

Lin YR, Schertz KF, Paterson AH (1995) Comparative analysis of QTLs affecting plant height and maturity across the Poaceae, in reference to an interspecific sorghum population. Genetics 141: 391–411

Melake-Berhan A, Hulbert SH, Butler LG, Bennetzen JL (1993) Structure and evolution of the genome of *Sorghum bicolor* and *Zea mays*. Theor Appl Genet 86: 598–604

Ming R, Liu SC, Lin YR, Da Silva J, Wilson W, Braga D, Van Deynze A, Wenslaff TF, Wu KK, Moore PH, Burnquist W, Sorrels ME, Irvine JE, Paterson AH (1998) Detailed alignment of Saccharum and Sorghum chromosomes: comparative organization of closely related diploid and polyploid genomes. Genetics 150: 1663–1682

Pereira MG, Lee M, Bramel-Cox P, Woodman W, Doebly J, Whitkus R (1994) Construction of an RFLP map in sorghum and comparative mapping in maize. Genome 37: 236–243

Tai PYP, Miller JD, Dean JL (1981) Inheritance of resistance to rust in sugarcane. Field Crop Res 4: 261–268

Tomkins JP, Yu Y, Miller-Smith H, Frisch DA, Woo SS, Wing R (1999) A bacterial artificial chromosome library for sugarcane. Theor Appl Genet 99: 419–424

Van Deynze AE, Nelson JC, Yglesias ES, Harrington SE, Braga DP, McCouch SR, Sorrells ME (1995) Comparative mapping in grasses. Wheat relationships. Mol Gen Genet 248: 744–754

Whitkus R, Doebley J, Lee M (1992) Comparative genome mapping of sorghum and maize. Genetics 132: 1119–1130

Xu GW, Magill CW, Schertz KF, Hart GE (1994) A RFLP linkage map of *Sorghum bicolor* (L.) Moench. Theor Appl Genet 89: 139–145

Zhang HB, Wing RA (1997) Physical mapping of the rice genome with BACs. Plant Mol Biol 35:115—12